PCT

WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

A11

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 5 :
C07C 323/62, 323/60, C07D 333/34
C07C 327/32, 317/50, 313/48
A61K 31/13, 31/38

A1

(11) International Publication Number: WO 90/05719

(43) International Publication Date: 31 May 1990 (31.05.90)

(21) International Application Number: PCT/GB89/01399

(22) International Filing Date: 23 November 1989 (23.11.89)

(30) Priority data:
8827305.7 23 November 1988 (23.11.88) GB

(71) Applicant *(for all designated States except US)*: BRITISH BIO-TECHNOLOGY LIMITED [GB/GB]; Watlington Road, Cowley, Oxford OX4 5LY (GB).

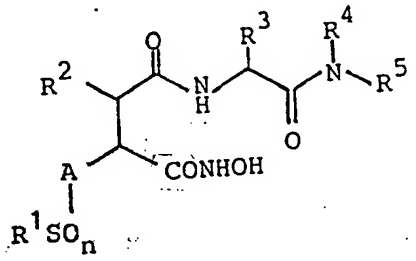
(72) Inventors; and
(75) Inventors/Applicants *(for US only)* : CAMPION, Colin [GB/GB]; 3 Howe Close, Wheatley, Oxon OX4 5LY (GB). DAVIDSON, Alan, Hornsby [GB/GB]; 27 Newland Mill, Witney, Oxon OX8 6HH (GB). DICKENS, Jonathan, Philip [GB/GB]; Burton House, Park Farm Road, High Wycombe, Bucks HP12 4AF (GB). CRIMMIN, Michael, John [GB/GB]; Oaklea, 64 Fernbank Road, Ascot SL5 8HE (GB).

(74) Agents: SHEARD, Andrew, Gregory et al.; Kilburn & Strode, 30 John Street, London WC1N 2DD (GB).

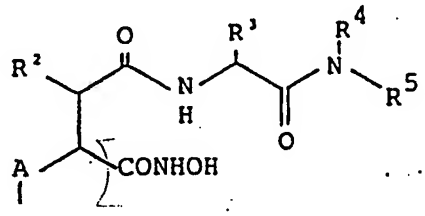
(81) Designated States: AT (European patent), AU, BE (European patent), CH (European patent), DE (European patent), DK, ES (European patent), FI, FR (European patent), GB (European patent), IT (European patent), JP, LU (European patent), NL (European patent), NO, SE (European patent), US.

Published
With international search report.
Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.

(54) Title: HYDROXAMIC ACID BASED COLLAGENASE INHIBITORS



(I)



(57) Abstract

Compounds of general formula (I), wherein R^1 represents hydrogen or an alkyl, phenyl, thiophenyl, substituted phenyl, phenylalkyl, heterocyclyl, alkylcarbonyl phenacyl or substituted phenacyl group; or, when n = 0, R^1 represents SR^X, wherein R^X represents a group (α); R^2 represents a hydrogen atom or an alkyl, alkenyl, phenylalkyl, cycloalkylalkyl or cycloalkenylalkyl group; R^3 represents an amino acid residue with R or S stereochemistry or an alkyl, benzyl, (C_1-C_6 alkoxy) benzyl or benzyloxy(C_1-C_6 alkyl) group; R^4 represents a hydrogen atom or an alkyl group; R^5 represents a hydrogen atom or a methyl group; n is an integer having the value 0, 1 or 2; and A represents a hydrocarbon chain optionally substituted with one or more alkyl, phenyl or substituted phenyl groups; and their salts and N-oxides are collagenase inhibitors and are useful in the management of disease involving tissue degradation and/or the promotion of wound healing. Diseases involving tissue degradation include arthropathy (particularly rheumatoid arthritis), inflammation, dermatological diseases, bone resorption diseases and tumour invasion.

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AT	Austria
AU	Australia
BB	Barbados
BE	Belgium
BF	Burkina Faso
BG	Bulgaria
BJ	Benin
BR	Brazil
CA	Canada
CF	Central African Republic
CG	Congo
CH	Switzerland
CM	Cameroon
DE	Germany, Federal Republic of
DK	Denmark
ES	Spain
FI	Finland
FR	France
GA	Gabon
GB	United Kingdom
HU	Hungary
IT	Italy
JP	Japan
KP	Democratic People's Republic of Korea
KR	Republic of Korea
LI	Liechtenstein
LK	Sri Lanka
LU	Luxembourg
MC	Monaco
MG	Madagascar
ML	Mali
MR	Mauritania
MW	Malawi
NL	Netherlands
NO	Norway
RO	Romania
SD	Sudan
SE	Sweden
SN	Senegal
SU	Soviet Union
TD	Chad
TG	Togo
US	United States of America

HYDROXAMIC ACID BASED COLLAGENASE INHIBITORS.

This invention relates to pharmaceutically and veterinarily active compounds, which are derivatives of hydroxamic acid.

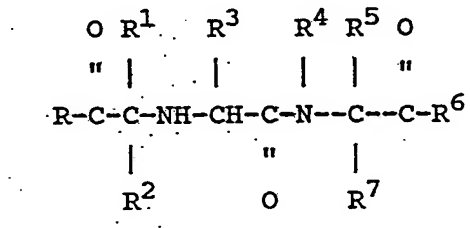
The compounds of the present invention act as inhibitors of metalloproteases involved in tissue degradation, such as collagenase, which initiates collagen breakdown, stromelysin (protoglycanase), gelatinase and collagenase (IV). There is evidence implicating collagenase as one of the key enzymes in the breakdown of articular cartilage and bone in rheumatoid arthritis (Arthritis and Rheumatism, 20, 1231 - 1239, 1977). Potent inhibitors of collagenase and other metalloproteases involved in tissue degradation are useful in the treatment of rheumatoid arthritis and related diseases in which collagenolytic activity is important. Inhibitors of metalloproteases of this type can therefore be used in treating or preventing conditions which involve tissue breakdown; they are therefore useful in the treatment of arthropathy, dermatological conditions, bone resorption, inflammatory diseases and tumour invasion and in the promotion of wound healing. Specifically, compounds of the present invention may be useful in the treatment of osteopenias such as osteoporosis, rheumatoid arthritis, osteoarthritis, periodontitis, gingivitis, corneal ulceration and tumour invasion.

A number of small peptide like compounds which inhibit metalloproteases have been described. Perhaps the most notable of these are those relating to the

angiotensin converting enzyme (ACE) where such agents act to block the conversion of the decapeptide angiotensin I to angiotensin II a potent pressor substance. Compounds of this type are described in EP-A-0012401.

Certain hydroxamic acids have been suggested as collagenase inhibitors as in US-A-4599361 and EP-A-0236872. Other hydroxamic acids have been prepared as ACE inhibitors, for example in US-A-4105789, while still others have been described as enkephalinase inhibitors as in US-A-4496540.

EP-A-0012401 discloses antihypertensive compounds of the formula:



wherein

R and R^6 are the same or different and are hydroxy, alkoxy, alkenoxy, dialkylamino alkoxy, acylamino alkoxy, acyloxy alkoxy, aryloxy, alkyloxy, substituted aryloxy or substituted aralkoxy wherein the substituent is methyl, halo, or methoxy, amino, alkylamino, dialkylamino, aralkylamino or hydroxyamino;

R^1 is hydrogen, alkyl of from 1 to 20 carbon atoms, including branched, cyclic and unsaturated alkyl groups;

substituted alkyl wherein the substituent is halo, hydroxy, alkoxy, aryloxy amino, alkylamino, dialkylamino, acrylamino, arylamino, guanidino, imidazolyl, indolyl, mercapto, alkylthio, arylthio, carboxy, carboxamido, carbalkoxy, phenyl, substituted phenyl wherein the substituent is alkyl, alkoxy or halo; aralkyl or heteroaralkyl, aralkenyl or heteroaralkenyl, substituted aralkyl, substituted heteroaralkyl, substituted aralkenyl or substituted hetereoaralkenyl, wherein the substituent is halor or dihalo, alkyl, hydroxy, alkoxy, amino, aminomethyl, acrylamino, dialkylamino, alkylamino, carboxyl, haloalkyl, cyano or sulphonamido, aralkyl or hetereoaralkyl substituted on the alkyl portion by amino or acylamino;

R^2 and R^7 are hydrogen or alkyl;

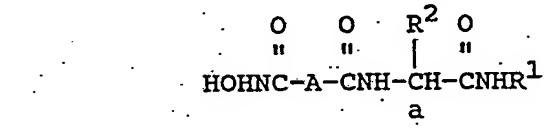
R^3 is hydrogen, alkyl, phenylalkyl, aminomethylphenylalkyl, hydroxyphenylalkyl, hydroxyalkyl, acetylaminoalkyl, acylaminoalkyl, acylaminoalkyl aminoalkyl, dimethylaminoalkyl, haloalkyl, guanidinoalkyl, imidazolylalkyl, indolylalkyl, mercaptoalkyl and alkylthioalkyl;

R^4 is hydrogen or alkyl;

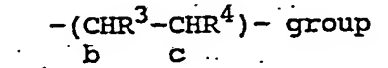
R^5 is hydrogen, alkyl, phenyl, phenylalkyl, hydroxyphenylalkyl, hydroxyalkyl, aminoalkyl, guanidinoalkyl, imidazolylalkyl, indolylalkyl, mercaptoalkyl or alkylthioalkyl;

R^4 and R^5 may be connected together to form an alkylene bridge of from 2 to 4 carbon atoms, an alkylene bridge of from 2 to 3 carbon atoms and one sulphur atom, an alkylene bridge of from 3 to 4 carbon atoms containing a double bond or an alkylene bridge as above, substituted with hydroxy, alkoxy or alkyl and the pharmaceutically acceptable salts thereof.

US-A-4599361 discloses compounds of the formula:



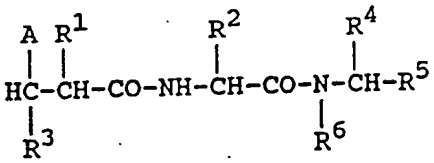
wherein
R^1 is C_1-C_6 alkyl;
R^2 is C_1-C_6 alkyl, benzyl, benzyloxybenzyl, (C_1-C_6 alkoxy)benzyl or benzyloxy(C_1-C_6 alkyl);
a is a chiral centre with optional R or S stereochemistry;
A is a



or a $-(CR^3=CR^4)-$ group wherein b and c are chiral centres with optional R or S stereochemistry;

R^3 is hydrogen, C_1-C_6 alkyl, phenyl or phenyl(C_1-C_6 alkyl) and R^4 is hydrogen, C_1-C_6 alkyl, phenyl(C_1-C_6 alkyl), cycloalkyl or cycloalkyl(C_1-C_6 alkyl).

EP-A-0236872 discloses generically compounds of the formula



wherein

A represents a group of the formula HN(OH)-CO- or HCO-N(OH)-;

R^1 represents a C_2-C_5 alkyl group;

R^2 represents the characterising group of a natural alpha-amino acid in which the functional group can be protected, amino groups may be acylated and carboxyl groups can be amidated, with the proviso that R^2 can not represent hydrogen or a methyl group;

R^3 represents hydrogen or an amino, hydroxy, mercapto, C_1-C_6 alkyl, C_1-C_6 alkoxy, C_1-C_6 acylamino, C_1-C_6-alkylthio, aryl-(C_1-C_6 alkyl)-, amino-(C_1-C_6-alkyl)-, hydroxy(C_1-C_6-alkyl)-, mercapto(C_1-C_6 alkyl) or carboxy(C_1-C_6 alkyl) group,

wherein the amino, hydroxy, mercapto or carboxyl groups can be protected and the amino groups may be acylated or the carboxyl groups may be amidated;

R^4 represents hydrogen or a methyl group;

R^5 represents hydrogen or a C_1-C_6 acyl, C_1-C_6 alkoxy-C_1-C_6 alkyl, di(C_1-C_6-alkoxy)methylene, carboxy, (C_1-C_6 alkyl)carbinyl, (C_1-C_6 alkoxy)carbinyl, arylmethoxy carbinyl, (C_1-C_6 alkyl)amino carbinyl or arylamino carbinyl group; and

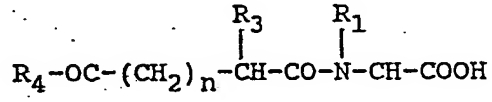
R^6 represents hydroxy or a methylene group; or

R^2 and R^4 together represent a group-$(CH_2)_n$-, wherein n represents a number from 4 to 11; or

R^4 and R^5 together represent a trimethylene group;

and pharmaceutically acceptable salts of such compounds, which are acid or basic.

US-A-4105789 generically discloses compounds which have the general formula



and salts thereof, wherein

R_1 is hydrogen, lower alkyl, phenyl lower alkylene, hydroxy-lower alkylene, hydroxyphenyl lower alkylene, amino-lower alkylene, guanidine lower

alkylene, mercapto-lower alkylene, lower alkyl-mercapto-lower alkylene, imidazolyl lower alkylene, indolyl-lower alkylene or carbamoyl lower alkylene;

R_2 is hydrogen or lower alkyl;

R_3 is lower alkyl or phenyl lower alkylene;

R_4 is hydroxy, lower alkoxy or hydroxyamino; and

n is 1 or 2.

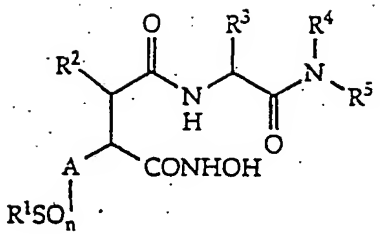
US-A-4496540 discloses compounds of the general formula:

A-B-NHOH

wherein A is one of the aromatic group-containing amino acid residues L-tryptophyl, D-tryptophyl, L-tyrosyl, D-tyrosyl, L-phenylalanyl, or D-phenylalanyl, and B is one of the amino acids glycine, L-alanine, D-alanine, L-leucine, D-leucine, L-isoleucine, or D-isoleucine; and pharmaceutically acceptable salts thereof.

It would however be desirable to improve on the solubility of known collagenase inhibitors and/or stomelysin inhibitors (whether as the free base or the salt) and, furthermore, increases in activity have also been sought. It is not a simple matter, however, to predict what variations in known compounds would be desirable to increase or even retain activity; certain modifications of known hydroxamic acid derivatives have been found to lead to loss of activity.

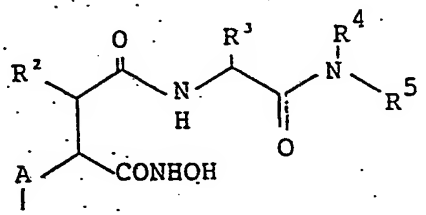
According to a first aspect of the invention, there is provided a compound of general formula I:



(I)

wherein:

R^1 represents a C_1-C_6 alkyl, phenyl, thiophenyl, substituted phenyl, phenyl(C_1-C_6)alkyl, heterocyclyl, (C_1-C_6)alkylcarbonyl, phenacyl or substituted phenacyl group; or, when n = 0, R^1 represents SR^X, wherein R^X represents a group:



;

R^2 represents a hydrogen atom or a C_1-C_6 alkyl, C_1-C_6 alkenyl, phenyl(C_1-C_6)alkyl, cycloalkyl(C_1-C_6)alkyl or cycloalkenyl(C_1-C_6)alkyl group;

R^3 represents an amino acid side chain or a C_1-C_6 alkyl, benzyl, (C_1-C_6 alkoxy)benzyl, benzyloxy(C_1-C_6 alkyl) or benzyloxybenzyl group;

R^4 represents a hydrogen atom or a C_1-C_6 alkyl group;

R^5 represents a hydrogen atom or a methyl group;

n is an integer having the value 0, 1 or 2; and

A represents a C_1-C_6 hydrocarbon chain, optionaly substituted with one or more C_1-C_6 alkyl, phenyl or substituted phenyl groups;

or a salt thereof.

Hereafter in this specification, the term "compound" includes "salt" unless the context requires otherwise.

As used herein the term "C_1-C_6 alkyl" refers to a straight or branched chain alkyl moiety having from one to six carbon atoms, including for example, methyl, ethyl, propyl, isopropyl, butyl, t-butyl, pentyl and hexyl, and cognate terms (such as "C^1-C^6 alkoxy") are to be construed accordingly.

The term "C_1-C_6 alkenyl" refers to a straight or branched chain alkyl moiety having one to six carbons and having in addition one double bond, of either E or Z stereochemistry where applicable. This term would include, for example, an alpha, beta-unsaturated methylene group, vinyl, 1-propenyl, 1- and 2-butenyl and 2-methyl-2-propenyl.

The term "cycloalkyl" refers to a saturated alicyclic moiety having from 3 to 8 carbon atoms and includes for example, cyclopropyl, cyclobutyl, cyclopentyl and cyclohexyl.

The term "cycloalkenyl" refers to an unsaturated alicycle having from 3 to 8 carbon atoms and includes cyclopropenyl, cyclobutenyl and cyclopentenyl, cyclohexenyl.

The term "substituted", as applied to a phenyl or other aromatic ring, means substituted with up to four substituents each of which independently may be C_1-C_6 alkyl, C_1-C_6 alkoxy, hydroxy, thiol, C_1-C_6 alkylthiol, amino, halo (including fluoro, chloro, bromo and iodo), triflouromethyl or nitro.

The term "amino acid side chain" means a characteristic side chain attached to the $-CH(NH_2)(COOH)$ moiety in the following R or S amino acids: glycine, alanine, valine, leucine, isoleucine, phenylalanine, tyrosine, tryptophan, serine, threonine, cysteine, methionine, asparagine, glutamine, lysine, histidine, arginine, glutamic acid and aspartic acid.

The term "hydrocarbon chain" includes alkylene, alkenylene and alkynylene chains of from 1 to 6 carbon atoms. Preferably the carbon atom of the hydrocarbon chain nearest to the hydroxamic acid group is a methylene carbon atom.

There are several chiral centres in the compounds according to the invention because of the presence of asymmetric carbon atoms. The presence of several asymmetreic carbon atoms gives rise to a number of diastereomers with the appropriate R or S stereochemistry at each chiral centre. General formula I and, where apprpriate, all other formulae in this

specification are to be understood to include all such stereoisomers and mixtures (for example racemic mixtures) thereof. Compounds in which the chiral centre adjacent the substituent R^3 has S stereochemistry and/or the chiral centre adjacent the substituent R^2 has R stereochemistry are preferred.

Further or other preferred compounds include those in which, independently or in any combination:

R^1 represents a hydrogen atom or a C_1-C_4 alkyl, phenyl, thiophenyl, benzyl, acetyl or benzoyl group;

R^2 represents a C_3-C_6 alkyl (for example isobutyl) group;

R^3 represents a benzyl or 4-(C_1-C_6)alkoxyphenylmethyl or benzyloxybenzyl group;

R^4 represents a C_1-C_4 alkyl (for example methyl) group; and

R^5 represents a hydrogen atom.

Particularly preferred compounds include:

1. [4-(N-Hydroxyamino)-2R-isobutyl-3S-(phenylthio-methyl)-succinyl]-L-phenylalanine-N-methylamide,

2. [4-(N-Hydroxyamino)-2R-isobutyl-3S-(thiophenyl-thio-methyl)succinyl]-L-phenylalanine-N-methylamide,

3. [4-(N-Hydroxyamino)-2R-isobutyl-3S-(benzylthiomethyl) succinyl]-L-phenylalanine-N-methylamide,

4. [4-(N-Hydroxyamino)-2R-isobutyl-3S-(acetylthiomethyl)succinyl]-L-phenylalanine-N-methylamide and

5. [4-(N-Hydroxyamino)-2R-isobutyl-3S-(thiolmethyl) succinyl]-L-phenylalanine-N-methylamide

6. [4-(N-Hydroxyamino)-2R-isobutyl-3S-(benzoylthiomethyl)succinyl]-L-phenylalanine-N-methylamide

7. [4-(N-Hydroxyamino)-2R-isobutyl-3S-(pivaloylthiomethyl)succinyl]-L-phenylalanine-N-methylamide

8. [4-(N-Hydroxyamino)-2R-isobutyl-3S-(phenylthiomethyl)succinyl]-L-phenylalanine-N-methylamide sodium salt

9. [4-(N-Hydroxyamino)-2R-isobutyl-3S-(4-methoxyphenyl-thiomethyl)succinyl]-L-phenylalanine-N-methylamide

10. [4-(N-Hydroxyamino)-2R-isobutyl-3S-(4-hydroxyphenylthiomethyl)succinyl]-L-phenylalanine-N-methylamide

11 [4-(N-Hydroxyamino)-2R-isobutyl-3S-(2-thiophenethiomethyl)succinyl]-L-phenylalanine-N-methylamide sodium salt

12. [4-(N-Hydroxyamino)-2R-isobutyl-3S-(4-methoxyphenylthiomethyl)succinyl]-L-phenylalanine-N-methylamide sodium salt

13. [4-(N-Hydroxyamino)-2R-isobutyl-3S-(4-tert-butylphenylthiomethyl)succinyl]-L-phenylalanine-N-methylamide

14. [4-(N-Hydroxyamino)-2R-isobutyl-3S-(2,4-dimethylphenylthiomethyl)succinyl]-L-phenylalanine-N-methylamide

15. bis-S,S'-([4(N-Hydroxyamino-2R-isobutyl-3S-(thiomethyl)succinyl]-L-phenylalanine-N-methylamide) disulphide

16. [4-(N-Hydroxyamino)-2R-isobutyl-3S-(3-bromophenylthio-methyl)succinyl]-L-phenylalanine-N-methylamide

17. [4-(N-Hydroxyamino)-2R-isobutyl-3S-(3-chlorophenylthiomethyl)succinyl]-L-phenylalanine-N-methylamide

18. [4-(N-Hydroxyamino)-2R-isobutyl-3S-(3-methylphenylthiomethyl)succinyl]-L-phenylalanine-N-methylamide

19. [4-(N-Hydroxyamino)-2R-isobutyl-3S-(4-(N-acetyl)-aminophenylthiomethyl)succinyl]-L-phenylalanine-N-methylamide

20. [4-(N-Hydroxyamino)-2R-isobutyl-3S-phenylsulphinylmethylsuccinyl]-L-phenylalanine-N-methylamide

21. [4-(N-Hydroxyamino)-2R-isobutyl-3S-phenylsulphonylmethylsuccinyl]-L-phenylalanine-N-methylamide

22. [4-(N-Hydroxyamino)-2R-isobutyl-3S-thiophenylsulphinylmethyl-succinyl]-L-phenylalanine-N-methylamide

23. [4-(N-Hydroxyamino)-2R-isobutyl-3S-thiophenylsulphonylmethyl-succinyl]-L-phenylalanine-N-methylamide

24. [4-(N-Hydroxyamino)-2R-isobutyl-3S-phenylsulphonylmethyl-succinyl]-L-phenylalanine-N-methylamide sodium salt

25. [4-(N-Hydroxyamino)-2R-isobutyl-3S-(4-(isobutyloxycarbonylamino)phenyl)thiomethyl-succinyl]-L-phenylalanine-N-methylamide

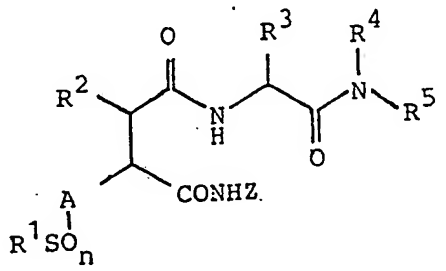
26. [4-(N-Hydroxyamino)-2R-isobutyl-3S-(4-(N-methyl-N-(tert-butoxycarbonyl)-glycylamino)phenyl)thiomethylsuccinyl]-L-phenylalanine-N-methylamide

and, where appropriate, their salts. Compounds 2 and 5 are especially preferred and compound 2 is the most preferred, because of its good collagenase-inhibiting and protoglycanase-inhibiting activities.

Compounds of general formula I may be prepared by any suitable method known in the art and/or by the following process, which itself forms part of the invention.

According to a second aspect of the invention, there is provided a process for preparing a compound of general formula I as defined above, the process comprising:

(a) deprotecting a compound of general formula II

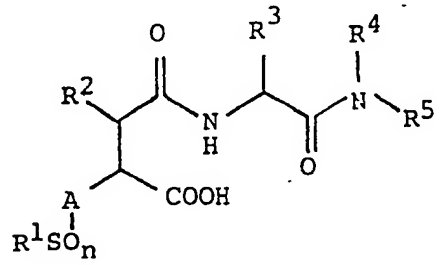


(II)

wherein:

R^1, R^2, R^3, R^4, R^5, A and n are as defined in general formula I and Z represents a protective group such as a benzyl group; or

(b) reacting a compound of general formula III



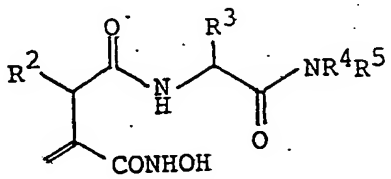
(III)

wherein:

R^1, R^2, R^3, R^4, R^5, A and n are as defined in general formula I,

with hydroxylamine or a salt thereof; or

(c) reacting a compound of general formula VIA



(VIA)

wherein

R^2, R^3, R^4 and R^5 are as defined in general formula I,

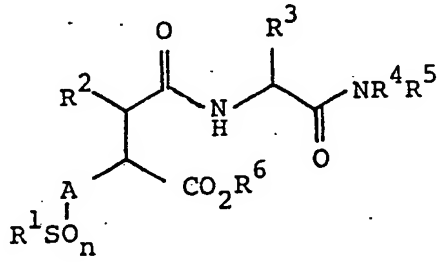
either with a thiol of the general formula R^1S, wherein R^1 is as defined in general formula I to give a compound of general formula I in which A represents a methylene group and n is 0,

or with a cuprate of the general formula $(R^1S\text{-}A^1)_2CuLi$, wherein R^1 is as defined in general formula I and A^1 is such that $-A^1-CH_2-$ is identical to -A-, as defined in general formula I.

(d) optionally after step (a), step (b) or step (c) converting a compound of general formula I into another compound of general formula I.

Compounds of general formula I which are sulphoxides or sulphones can be derived from thiol compounds of general formula I by oxidation. Alternatively, thiols of general formula II or III may be oxidised. Compounds of general formula I which are disulphides (ie compounds wherein R^1 represents SR^X) may be derived from thiol esters of general formula I by milk oxidation, for example in air.

A compound of general formula II may be prepared from a compound of general formula III by reaction with an O-protected (such as benzyl) hydroxylamine. A compound of general formula III may be prepared by desterification (such as hydrolysis) of an ester of the general formula IV

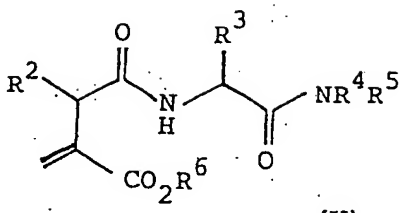


(IV)

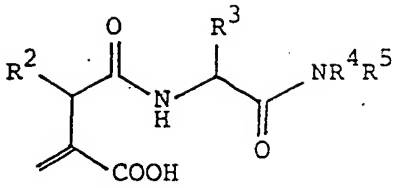
wherein:

R^1, R^2, R^3, R^4, R^5, A and n are as defined in general formula I and R^6 represents C_1-C_6 alkyl, phenyl C_1-C_6 alkyl or substituted phenyl C_1-C_6 alkyl.

A compound of general formula IV can be prepared from an ester of general formula V or an acid of general formula VI



(V)



(VI)

wherein:

R^2, R^3, R^4 and R^5 are as defined in general formula I and R^6 represents C_1-C_6 alkyl, phenyl C_1-C_6 alkyl or substituted phenyl C_1-C_6 alkyl

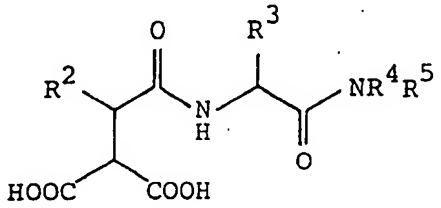
by reaction with a thiol R^1SH, wherein R^1 is as defined in general formula I, to give compounds wherein A represents a methylene group,

or by reaction with a cuprate of the general formula $(R^1S\text{-}A^1)_2CuLi$, wherein R^1 is as defined in general formula I and A^1 is such that $-A^1-CH_2-$ is identical to -A-, as defined in general formula I.

Esters of general formula V can be prepared by esterifying acids of general formula VI with an appropriate alcohol R^6OH or other esterifying agent.

Compounds of general formula VIA can be prepared by reacting compounds of general formula VI with hydroxylamine or a salt thereof.

An acid of general formula VI can be prepared by reacting a malonic acid derivative of general formula VII



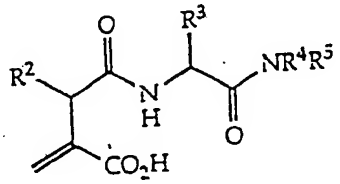
(VII)

wherein:

R^2, R^3, R^4 and R^5 are as defined in general formula I

with formaldehyde in the presence of pyridine.

An acid of general formula VII can in turn be prepared by desterifying (for example hydrolysing) a compound of general formula VIII

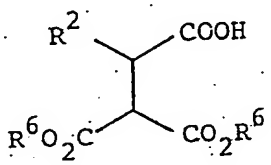


(VIII)

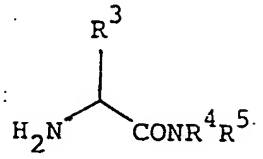
wherein:

R^2, R^3, R^4 and R^5 are as defined in general formula I and R^6 represents C_1-C_6 alkyl, phenyl C_1-C_6 alkyl or substituted phenyl C_1-C_6 alkyl.

A compound of general formula VIII can be prepared by reacting a compound of general formula IX with a compound of general formula X



(IX)



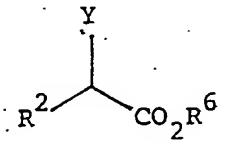
(X)

wherein:

R^2, R^3, R^4 and R^5 are as defined in general formula I and R^6 represents C_1-C_6 alkyl, phenyl C_1-C_6 alkyl or substituted phenyl C_1-C_6 alkyl.

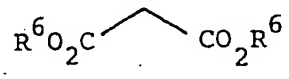
The starting materials and other reagents are either available commercially or can be synthesised by simple chemical procedures.

For example, a substituted acid of general formula IX may be prepared by reacting an ester of the general formula XI



(XI)

wherein Y represents halo and R^5 is as defined above and R^2 and R^6 as defined above, with a malonate derivative of the general formula XII



(XII)

wherein R^6 is as defined above with the proviso that when R^6 is aromatic in general formula XI it is aliphatic in general formula XII or vice versa, and selectively de-esterifying.

Compounds of general formula XI can simply be derived from amino acids, which can be obtained in enantiomerically pure form, enabling a choice of optically active compounds of general formula I to be prepared.

Compounds of general formulae II and III are valuable intermediates in the preparation of compounds of general formula I. According to a third aspect of the invention, there is therefore provided a compound of general formula II. According to a fourth aspect of the invention, there is provided a compound of general formula III.

As mentioned above, compounds of general formula I are useful in human or veterinary medicine as they are active inhibitors, of metalloproteases involved in tissue degradation.

According to a fifth aspect of the invention, there is provided a compound of general formula I for use in human or veterinary medicine, particularly in the management (by which is meant treatment of prophylaxis) of disease involving tissue degradation, in particular rheumatoid arthritis, and/or in the promotion of wound healing.

According to a sixth aspect of the invention, there is provided the use of a compound of general formula I in the preparation of an agent for the management of disease involving tissue degradation, particularly rheumatoid arthritis, and/or in the promotion of wound healing. Compounds of general formula I can therefore be used in a method of treating disease involving tissue degradation, particularly rheumatoid arthritis, and/or in a method of promoting wound healing, the method in either case comprising administering to a human or animal patient an effective amount of a compound of general formula I.

The potency of compounds of general formula I to act as inhibitors of collagenase (a metalloprotease involved in tissue degradation) was determined by the procedure of Cawston and Barrett, (Anal. Biochem., 99, 340-345, 1979) and their potency to act as inhibitors of stromelysin was determined using the procedure of Cawston et al (Biochem. J., 195, 159-165 1981), both of which techniques are to be described more fully in the examples and are incorporated by reference herein so far as the law allows.

According to a seventh aspect of the invention, there is provided a pharmaceutical or veterinary formulation comprising a compound of general formula I and a pharmaceutically and/or veterinarily acceptable carrier. One or more compounds of general formula I may be present in association with one or more non-toxic pharmaceutically and/or veterinarily acceptible carriers and/or diluents and/or adjuvents and if desired other active ingredients.

According to an eighth aspect of the invention, there is provided a process for the preparation of a pharmaceutical or veterinary formulation in accordance with the seventh aspect, the process comprising admixing a compound of general formula I and a pharmaceutically and/or veterinarily acceptable carrier.

Compounds of general formula I may be formulated for administration by any route and would depend on the disease being treated. The compositions may be in the form of tablets, capsules, powders, granules, lozenges, liquid or gel preparations, such as oral, topical, or sterile parental solutions or suspensions.

Tablets and capsules for oral administration may be in unit dose presentation form, and may contain conventional excipients such as binding agents, for example syrup, acacia, gelatin, sorbitol, tragacanth, or polyvinyl-pyroilidone; fillers for example lactose, sugar, maize-starch, calcium phosphate,sorbitol or glycine; tabletting lubricant, for example magnesium sterate, talc, polyethylene glycol or silica; disintegrants, for example potato starch, or acceptable wetting agents such as sodium lauryl sulphate. The tablets may be coated according to methods well known in normal pharmaceutical practice. Oral liquid preparations may be in the form of, for example, aqueous or oily suspensions, solutions, emulsions, syrups or elixirs, or may be presented as a dry product for reconstitution with water or other suitable vehicle before use. Such liquid

preparations may contain coventional additives such as suspending agents, for example sorbitol, syrup, methyl cellulose, glucose syrup, gelatin, hydrogenated edible fats; emulsifiying agents, for example lecithin, sorbitan monooleate, or acacia; non-aqujeous vehicles (which may include edible oils), for example almond oil, fractionated coconut oil, oily esters such as glycerine, propylene glycol, or ethyl alcohol; preservatives, for example methyl or propyl p-hydroxybenzoate or sorbic acid, and if desired conventional flavouring or colouring agents.

The dosage unit involved in oral administration may contain from about 1 to 250 mg, preferably from about 25 to 250 mg of a compound of general formula I. A suitable daily dose for a mammal may vary widely depending on the condition of the patient. However, a dose of a compound of general formula I of about 0.1 to 300mg/kg body weight, particularly from about 1 to 100 mg/kg body weight may be appropriate.

For topical application to the skin the drug may be made up into a cream, lotion or ointment. Cream or ointment formulations that may be used for the drug are conventional fomulations well known in the art, for example, as described in standard text books of pharmaceutics such as the British Pharmacopoeia.

For topical applications to the eye, the drug may be made up into a solution or suspension in a suitable sterile aqueous or non-aqueous vehicle. Additives, for instance buffers such as sodium metabisulphite or disodium edeate; preservatives including bactericidal

and fungicidal agents, such as phenyl mercuric acetate or nitrate, benzalkonium chloride or chlorohexidine, and thickening agents such as hypromellose may also be included.

The dosage employed for the topical administration will, of course, depend on the size of the area being treated. For the eyes each dose will be typically in the range from 10 to 100 mg of the compound of general formula I.

The active ingredient may also be administered parenterally in a sterile medium. The drug depending on the vehicle and concentration used, can either be suspended or dissolved in the vehicle. Advantageously, adjuvants such as a local anasthetic, preservative and buffering agents can be dissolved in the vehicle.

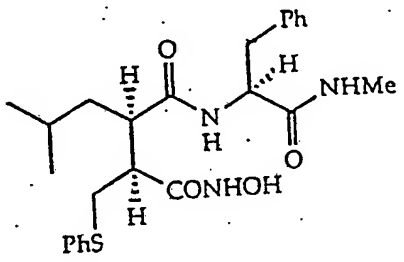
For use in the treatment of rheumatoid arthritis the compounds of this invention can be administered by the oral route or by injection intra-articularly into the affected joint. The daily dosage for a 70 kg mammal will be in the range of 10 mgs to 1 gram of a compound of general formula I.

The following examples illustrate the invention, but are not intended to limit the scope in any way. The following abbreviations have been used in the Examples:-

DCC - Dicyclohexylcarbodiimide
DCM - Dichloromethane
DCU - Dicyclohexylurea
DIPE - Diisopropyl ether
DMF - N,N-dimethylformamide
HOBT - Hydroxybenztriazole
NMM - N-Methylmorpholine
TFA - Trifluoroacetic acid
THF - Tetrahydrofuran
WSCDI - N-(Dimethylaminoethyl)-N'-ethylcarbodiimide

Example 1

[4-(N-Hydroxyamino)-2R-isobutyl-3S-(phenylthiomethyl)-succinyl]-L-phenylalanine-N-methylamide



a) 2R-Bromo-5-methylpentanoic acid.

D-Leucine (100g, 0.76 mol) and potassium bromide (317.5g, 2.67 mol) were dissolved in aqueous acid (150ml concentrated sulphuric acid in 500ml of water). The solution was cooled to -2° and sodium nitrite (69.6g, 0.95 mol in water) was added over 1h taking care to maintain the temperature between -1 and -2°. After addition was complete the mixture was kept at 0° for a further hour, then DCM was added and the mixture stirred for a few minutes. The layers were separated

and the aqeous phase was washed with further portions of DCM (5 x 250ml). The combined organic layers were dried over magnesium sulphate then the solvent removed to give the acid as a pale yellow oil (123.1g, 0.63 mol, 83%)

$[alpha]_D$ = +38.0° (c = 2, methanol)

$delta_H$ (250 MHz, $CDCl_3$) 4.29 (1H, t, J= 6.5Hz, $BrC\underline{H}CO_2H$), 1.91 (2H, t, J= 7Hz, $CHC\underline{H}_2CH$), 1.83 (1H, m, $Me_2C\underline{H}$), and 0.94 (6H, 2xd, J= 7Hz, $(C\underline{H}_3)_2CH$)

b) tert-Butyl 2R-Bromo-5-methylpentanoate.

2R-Bromo-5-methylpentanoic acid (123g, 0.63 mol) was dissolved in DCM (400ml) and the solution cooled to -40° while isobutene was condensed in to roughly double the volume. Maintaining the temperature at -40° concentrated sulphuric acid (4ml) was added dropwise. When the addition was complete the reaction was allowed to warm to room temperature overnight. The resultant solution was concentrated to half the volume by removing the solvent at reduced pressure, then the DCM was washed twice with an equal volume of 10% sodium bicarbonate solution. The organic layer was dried over magnesium sulphate and the solvent removed under reduced pressure to leave the title compound as a yellow oil (148.0g, 0.59 mol, 94%).

$[alpha]_D$ = +23.0° (c = 2, methanol)

delta$_H$ (250 MHz, $CDCl_3$) 4.18 (1H, t, J= 6.5Hz, $BrC\underline{H}CO_2H$), 1.89 (2H, m, $CHC\underline{H}_2CH$), 1.78 (1H, m, $Me_2C\underline{H}$), 1.49 (9H, s, $(CH_3)_3C$) and 0.94 (6H, 2xd, J= 7Hz, $(CH_3)_2CH$)

delta$_C$ (63.9 MHz, $CDCl_3$) 167.0, 82.0, 46.3, 43.4, 27.6, 26.3, 22.2, and 21.6.

c) Benzyl (2-benzloxycarbonyl-3R-(tert-butoxycarbonyl)-5-methylhexanoate.

Dibenzyl malonate (124.5g, 0.44 mol) was taken up in dry DMF and potassium tert-butoxide (49.2g, 0.44 mol) was added portionwise with stirring and cooling. When a homogeneous solution had formed it was cooled to 0° then tert-butyl-2R-bromo-5-methylpentanoate (110.0g, 0.44 mol) in DMF (200 ml) was added dropwise over 1h. When addition was complete the reaction was transfered to a cold room at <5° and left for 4 days. The reaction mixture was partitioned between ethyl acetate and saturated ammonium chloride then the aqueous layer extracted with further ethyl acetate (4x500ml), drying and solvent removal left an oil (228g) heavily contaminated with DMF. This oil was taken into ether (1 litre) and washed with brine (2x1l) then the organic layer dried (magnesium sulphate), solvent removed under reduced pressure to leave the desired material (179g) contaminated with a small amount of dibenzyl malonate.

$[alpha]_D = +22.5^o$ (c = 2, methanol)

delta$_H$ (250 MHz, $CDCl_3$) 7.40 - 7.25 (10H, m, Aromatic H), 5.14 (4H, 2xABq, $C\underline{H}_2Ph$), 3.77 (1H, d, J= 10Hz, $BnO_2CC\underline{H}CO_2Bn$), 3.09 (1H, dt, J= 10,6Hz, $CH_2C\underline{H}CO_2tBu$), 1.50 (3H, m, CH_2 + $C\underline{H}Me_2$)1.41 (9H, s, $C(C\underline{H}_3)_3$) and 0.88 (6H, 2xd, J= 7Hz).

d) [4-Benzyloxy-3-benzyloxycarbonyl-2R-isobutyl-succinyl]-L-phenylalanine-N-methylamide

Benzyl(2-benzyloxycarbonyl-5-methyl-3R-tert-butoxycarbonyl)-hexanoate (281.4g, 0.56 mol) was taken up in 5% water in TFA (410 ml) and allowed to stand at 5^o overnight. After this time the TFA was evaporated under reduced pressure then the residue partitioned between DCM (1l) and brine (200ml). Solvent removal left an oil which crystallised on standing (230g).

The crude acid from this reaction was dissolved in DMF (1l), then HOBT (95.3g, 0.64 mol), NMM (64g, 0.64 mol) and phenylalanine-N-methylamide (113.0g, 0.64 mol) were added at room temperature. The mixture was cooled to 0^o before dropwise addition of DCC (131.0g, 0.64 mol) in THF (1l). This solution was stirred to room temperature over the weekend. The precipitated DCU was removed by filtration then the solvents were removed from the filtrate under reduced pressure to leave an oil. This oily residue was dissolved in ethyl acetate then washed with 10% citric acid, 10% sodium bicarbonate and saturated brine. The organic layer was dried (magnesium sulphate), filtered then the solvent removed under reduced pressure to give the title compound as an oil (400g). This material was columned on silica using gradient elution (0 - 50% ethyl

acetate in hexane) to remove impurities and separate a small amount of the minor diastereoisomer. The material from the column (195g) was recrystallised from DIPE to give the title compound as a white crystalline solid (140.2g, 0.25 mol, 47%)

m.p. 98 -99°

Analysis calculated for $C_{33}H_{38}N_2O_6$

Requires C 70.95 H 6.86 N 5.01

Found C 70.56 H 6.89 N 5.06

$delta_H$ (250MHz, $CDCl_3$) 7.42 - 7.13 (15H ,m, Aromatic H), 6.58 (1H, d, J=7.7Hz, CONH), 5.75 (1H, m, CONHMe), 5.20 - 5.05 (4H, m, OCH_2Ph), 4.50 (1H, dt, J= 6.9,7.7Hz, $CHCH_2Ph$), 3.79 (1H, d, J= 9.1Hz, $CH(CO_2Bn)$), 3.15 - 2.91 (2H, m, CH_2Ph), 2.65 (3H, d, J= 4.8Hz, $CONHCH_3$), 1.52 (1H, m, $CHCH_2CH$), 1.32 (1H, m, $CH(CH_3)$), 1.05 (1H, m, $CHCH_2CH$), and 0.74 (6H, 2xd, J= 6.5Hz, $CH(CH_3)_2$)

e) [4-Hydroxy-2R-isobutyl-3-ethenylsuccinyl]-L-phenylalanine-N-methylamide.

[4-Benzyloxy-3-benzyloxycarbonyl-2R-isobutylsuccinyl]-L-phenylalanine-N-methylamide (29.6g, 53mmol) was taken up in ethanol, ammonium formate (16.7g, 265mmol) added followed by 10% palladium on charcoal (6g) as a slurry in isopropyl alcohol. After 30 minutes at room temperature the catalyst was removed by filtration, then washed with ethanol to give a solution of the crude diacid. To this was added piperidine (5.0g) and the mixture stirred at room temperature for 15 minutes before addition of aqueous formaldehyde (40%

solution, 25ml). After 18 hours at room temperature the mixture was refluxed for 1 h. Solvents were removed under reduced pressure and the residue partitioned between ethyl acetate and citric acid. The acid layer was extracted with further portions of ethyl acetate (2x250ml), the combined organic layers were extracted with potassium carbonate (3x200ml). These base extracts were acidified to pH 4 and re-extracted with DCM then the organic layer dried over magnesium sulphate. Solvent removal under reduced pressure gave the desired product as a white solid (9.35g, 27.0mmol, 51%).

m.p. 149-151°C

$delta_H$ (250MHz, $CDCl_3$) 8.37 (2H, d, J= 9.0Hz, CONH), 7.39 (1H, m, CONHMe), 7.27 - 7.06 (5H, m, Aromatic H), 6.40 (1H, s, CH_2CHCO_2H), 5.78 (1H, s, CH_2CHCO_2H), 4.93 (1H, q, J= 7Hz, $CHCH_2Ph$), 3.92 (1H, m, $CH_2CHCONH$), 2.95 (2H, m, CH_2Ph), 2.71 (3H, d, J= 4.1Hz, $NHCH_3$), 1.68 (1H, m), 1.45 (2H, m), and 0.86 (6H, 2xd, J= 5.8Hz, $CH(CH_3)_2$).

$delta_C$ (63.9Hz, $CDCl_3$) 173.3, 172.8, 169.6, 139.1, 136.3, 129.2, 128.3, 127.0, 126.6, 54.4, 43.5, 41.4, 39.1, 26.2, 25.7, 22.5 and 22.4

f) [4-Hydroxy-2R-isobutyl-3S-(phenylthiomethyl)-succinyl]-L-phenylalanine-N-methylamide

[4-Hydroxy-2R-isobuty-3-ethenylsuccinyl]-L-phenyl-alanine-N-methylamide (15.0g, 44mmol) was dissolved in thiophenol

(150ml) and the mixture stirred in the dark under nitrogen at 60° for 2 days. Ether was added to the cooled reaction mixture and the precipitated product collected by filtration. The solid was washed with large volumes of ether and dried under vacuum to give the title compound (13.1g, 28.7mmol, 65%).

m.p. 199-201°C

Analysis calculated for $C_{25}H_{32}N_2O_4S$

Requires C 65.76 H 7.06 N 6.14 S 7.02

Found C 65.69 H 7.06 N 6.07 S 7.05

$delta_H$ (250MHz, D_6-DMSO) 8.40 (1H, d, J= 9Hz, CONH), 7.82 (1H, m, CONHMe), 7.35 - 7.10 (7H, m, Aromatic H), 7.04 (3H, m, Aromatic H), 4.62 (1H, m, $CHCH_2Ph$), 2.94 (1H, dd, J= 14,5Hz, $CHCH_2Ph$), 2.89 (1H, dd, J= 14,9Hz, $CHCH_2Ph$), 2.62 (3H, d, J= 4.5Hz, $CONHCH_3$), 2.41 (3H, m, 2xCH + CH_2SPh), 2.23 (1H, d, J= 12Hz, CH_2SPh), 1.43 (1H, m, $CHCH_2CH$), 1.30 (1H, bm, $CH(CH_3)_2$), 0.90 (1H, m, $CHCH_2CH$) and 0.78 (6H, 2xd, J= 6.5Hz, $CH(CH_3)_2$.

g) [4-(N-Hydroxyamino)-2R-isobutyl-3S-(phenylthiomethyl) succinyl]-L-phenylalanine-N-methylamide

[4-Hydroxy-2R-isobutyl-3S-(phenylthiomethyl)succinyl]-L-phenylalanine-N-methylamide (16.8g, 37 mmol) and HOBT (6.6g, 44 mmol) were dissolved in DCM / DMF (4:1) and the mixture cooled to 0° before adding WSCDI (8.5g, 44 mmol) and NMM (4.5g, 44 mmol). The mixture was stirred at 0° for 1h to ensure complete formation of the activated ester. Hydroxylamine hydrochloride (3.8g, 55 mmol) and NMM (5.6g, 55 mmol) were dissolved in DMF then this mixture added dropwise to the cooled

solution of the activated ester. After 1h the reaction was poured into ether / water (1:1) whereupon the desired product precipitated as white crystals. These were collected by filtration, further washed with ether and water then dried under vacuum at 50°. This material was recrystallised from methanol / water (1:1) to remove a trace of the minor diastereomer (9.03g, 19.2 mmol, 52%).

m.p. 227-229°C

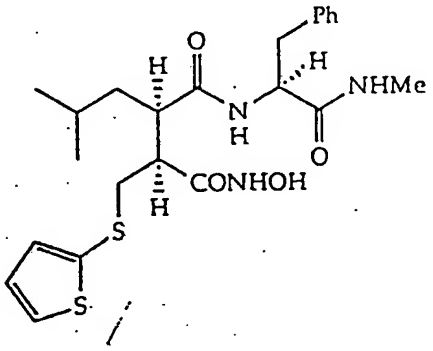
$[alpha]_D$ = -88° (c = 10 , methanol)

$delta_H$ (250MHz, D_6-DMSO) 8.84 (1H, d, J= 1.5Hz, NHOH), 8.35 (1H, d, J= 8.7Hz, CONH), 7.87 (1H, m, CONHMe), 7.29 - 6.92 (11H, m, Aromatic H + NHOH), 4.60 (1H, m, $CHCH_2Ph$), 2.94 (1H, dd, J= 13.5,4.3, $CHCH_2Ph$), 2.77 (1H, dd, J= 13.5,10, $CHCH_2Ph$), 2.60 (3H, d,J= 4.6Hz), 2.53 (1H, m), 2.41 (1H, m), 2.20 (1H, dd, J= 13.4,2.2Hz, CH_2SPh), 2.09 (1H, dd, J=13.4,2.4Hz, CH_2SPh), 1.38 (2H, m, $CHMe_2$ + $CHCH_2CH$), 0.88 (1H, m, $CHCH_2CH$), 0.82 (3H, d, J= 6.4Hz, $CH(CH_3)_2$), and 0.74 (3H, d, J+ 6.4Hz, $CH(CH_3)_2$).

$delta_C$ (63.9MHz, D_6-DMSO) 172.9, 171.6, 166.3, 138.1, 136.7, 129.1, 128.9, 128.0, 127.3, 126.4, 125.2, 54.2, 46.4, 46.0, 37.7, 32.4, 25.6, 25.2, 24.2, and 21.7.

Example 2

[4-(N-Hydroxyamino)-2R-isobutyl-3S-(thiophenylthiomethyl) succinyl]-L-phenylalanine-N-methylamide



a) [4-N-Hydroxy-2R-isobutyl-3S-(thiophenylthiomethyl) succinyl]-L-phenylalanine-N-methylamide

The title compound was prepared from [4-Hydroxy-2R-isobutyl-3-ethenylsuccinyl]-L-phenylalanine-N-methylamide (400mg, 1.16mmol) by the method described in example 1f, substituting thiophenethiol in the place of thiophenol to give a material (320mg, 0.73mmol, 63%) with the following characteristics.

m.p. 184-186^{o}C

delta$_{H}$ (250MHz, D_6-DMSO) 8.29 (1H, d, J= 8.1Hz, CONH), 7.84 (1H, m, CONHMe), 7.57 (1H, d, J= 5.1Hz, Thiophene H), 5H, m, Aromatic H), 7.00 (2H, m, Thiophene H), 4.50 (1H, m, $CHCH_2Ph$), 2.91 (1H, m, $CHCH_2Ph$), 2.75 (1H, m, $CHCH_2Ph$), 2.56 (3H, d, J= 4.0Hz, $CONHCH_3$), 2.34 (3H, m), 1.99 (1H, d, J= 9.3Hz,

$C\underline{H}_2SHet$), 1.42 (1H, m, $CHC\underline{H}_2CH$), 1.29 (1H, bm, $C\underline{H}(CH_3)_2$), 0.87 (1H, m, $CHC\underline{H}_2CH$), 0.79 (3H, d, J= 6.4Hz, $CH(C\underline{H}_3)_2$), and 0.72 (3H, d, J= 6.4Hz, $CH(C\underline{H}_3)_2$).

b) [4-(N-Hydroxyamino)-2R-isobutyl-3S-(thiophenylthiomethyl)succinyl]-L-phenylalanine-N-methylamide

Prepared by the method described in example 1g to give material with the following characteristics

m.p. 236-238°C

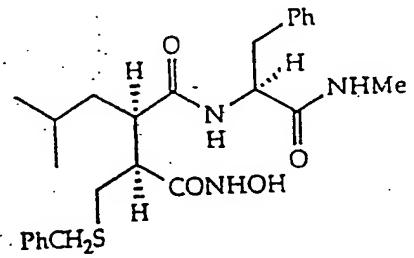
Analysis calculated for $C_{23}H_{30}N_2O_4S_2$
Requires C 57.84 H 6.54 N 8.80
Found C 57.64 H 6.48 N 8.85

$delta_H$ (250MHz, D_6-DMSO) 8.80 (1H, s, $CONHO\underline{H}$), 8.08 (1H, d, J=8Hz, $CON\underline{H}$), 7.52 (1H, m, $CON\underline{H}Me$), 7.32 (1H, dd, J= 4.6,2.9Hz, Thiophene H), 7.17 - 6.95 (5H, m, Aromatic H), 6.89 (2H, m, Thiophene H), 4.46 (1H, m, $CHC\underline{H}_2Ph$), 2.89 (1H, dd, J=13.6,4.4Hz, $CHC\underline{H}_2Ph$), 2.72 (1H, dd, J= 13.6,10.5Hz, $CHC\underline{H}_2Ph$), 2.54 (3H, d, J= 4.3Hz, $CONHC\underline{H}_3$), 2.46 (1H, d, J= 12.1Hz, $C\underline{H}_2S$), 2.35 (1H, bt, J= 10.2Hz), 2.14 (1H, bt, J= 10.2Hz), 1.98 (1H, dd, J=12.7,2.5Hz, $CHC\underline{H}_2Ph$), 1.35 (1H, bt, J= 11.4Hz, $CHC\underline{H}_2CH$), 1.22 (1H, bm, $CH(C\underline{H}_3)_2$), 0.86 (1H, bt, J=12.6Hz, $CHC\underline{H}_2CH$), 0.74 (3H, d, J= 6.3Hz, $CH(C\underline{H}_3)_2$), and 0.68 (3H, d, J= 6.4Hz, $CH(C\underline{H}_3)_2$).

$delta_C$ (63.9MHz, D_6-DMSO) 172.5, 171.6, 166.1, 138.0, 133.8, 132.7, 129.4, 129.2, 128.1, 127.8, 126.5, 54.2, 46.2, 46.0, 38.5, 37.6, 25.8, 25.2, 24.2, and 21.7.

Example 3

[4-(N-Hydroxyamino)-2R-isobutyl-3S-(benzylthiomethyl) succinyl]-L-phenylalanine-N-methylamide



Prepared by the method described in example 1g to give material with the following characteristics

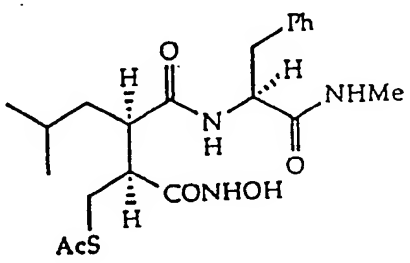
m.p. °

Analysis calculated for $C_{27}H_{37}N_3O_5S.0.5H_2O$
Requires C 61.81 H 7.30 N 8.00
Found C 61.85 H 7.15 N 7.45

$delta_H$ (250MHz, D_6-DMSO) 8.40 (1H, s, CONHOH), 8.22 (1H, m, NHMe), 7.20 (5H, m, Aromatic H), 6.58 (4H, m), 4.10 (1H, m, $CHCH_2Ph$), 3.22 (3H, s, OCH_3), 3.04 - 2.45 (4H, m, $2xCH_2Ar$), 2.42 (3H, d, J= 6Hz, $NHCH_3$), 2.32 - 2.08 (4H, m), 0.78 (2H, m, $CHCH_2CH$), and 0.40 - 0.18 (7H, m, $(CH_3)_2CH$).

Example 4

[4-(N-Hydroxyamino)-2R-isobutyl-3S-(acetylthiomethyl) succinyl]-L-phenylalanine-N-methylamide



Prepared by the method described in example 1g to give material with the following characteristics

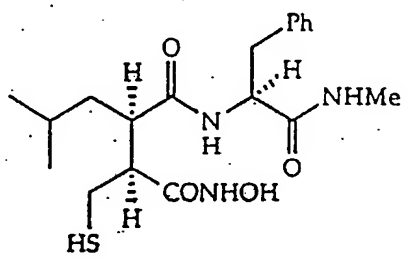
m.p. 226-227°C

Analysis calculated for $C_{21}H_{31}N_3O_5S.H_2O$
Requires C 55.37 H 7.30 N 9.22
Found C 55.57 H 6.99 N 9.53

$delta_H$ (250MHz, D_6-DMSO) 8.84 (1H, s, NHOH), 8.36 (1H, d, J= 8Hz, CONH), 7.80 (1H, d, J= 6Hz, NHMe), 7.20 (%h, m, Aromatic H), 4.58 (1H, m, $CHCH_2Ph$), 3.16 - 2.62 (2H, m, $CHCH_2Ph$), 2.54 (3H, d, J= 4Hz, $NHCH_3$), 2.22 (3H, s, CH_3COS), 2.36 - 2.10 (4H, m, $CHCHCH_2S$), 1.36 (2H, m, $CHCH_2CH$), and 0.98 - 0.66 (7H, m, $CH(CH_3)_2$).

Example 5

[4-(N-Hydroxyamino)-2R-isobutyl-3S-(thiolmethyl) succinyl]-L-phenylalanine-N-methylamide



[4-(N-Hydroxyamino)-2R-isobutyl-3S-(acetylthiomethyl) succinyl]-L-phenylalanine-N-methylamide (30mg, 0.06mmol) was stirred in methanol (3ml) with methylamine (1ml methanolic solution) at room temperature. After 30 minutes the crystalline product (20mg, 0.05mmol, 74%) was filtered off and dried.

m.p. 234°C

Analysis calculated for $C_{19}H_{39}N_3O_4S.1.5H_2O$

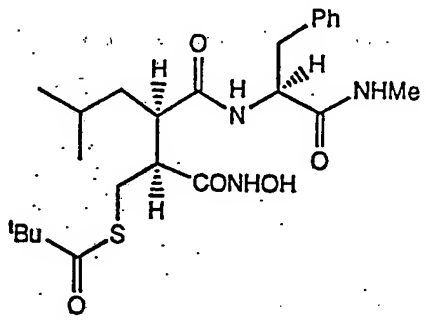
Requires C 54.10 H 7.63 N 9.94 S 7.60

Found C 54.28 H 7.16 N 10.43 S 7.80

$delta_H$ (250MHz, D_6-DMSO) 8.28 (1H, d, J= 9Hz, NHOH), 7.80 (1H, m, NHMe), 7.22 (5H, m, Aromatic H), 4.60 (1H, m, $CHCH_2Ph$), 3.08 - 2.56 (2H, m, $CHCH_2Ph$), 2.50 (3H, d, J= 4Hz, $NHCH_3$), 2.40 - 2.02 (4H, m, $CHCHCH_2SH$), 1.44 - 1.22 (2H, m, $CHCH_2CH$) and 0.98 - 0.72 (7H, m, $CH(CH_3)_2$).

Example 7

[4-(N-Hydroxyamino)-2R-isobutyl-3S-(pivaloylthiomethyl) succinyl]-L-phenylalanine-N-methylamide



[4-Hydroxy-2R-isobutyl-3S-(pivaloylthiomethyl) succinyl]-L-phenylalanine-N-methylamide (0,8g, 1.7 mmol) and HOBT (0.31g, 2.1 mmol) were dissolved in 1:1 DCM/DMF and the mixture cooled to 0°C before adding WSDCI (0.4g, 2.1mmol) and NMM (0.21g, 2.1mmol). The mixture was stirred at 0°C for 1h to ensure complete formation of the activated ester. Hydroxylamine hydrochloride (0.18g, 2.6mmol) and NMM (0.26g, 2.6mmol) were dissolved in DMF then this mixture was added dropwise to the cooled solution of the activated ester. After 1h the reaction was poured into ether/water (1:1) whereupon the desired product precipitated as white crystals. These were collected by filtration, further washed with ether and water, then dried under vacuum at 50°C. This material was recrystallised from methanol/water (1:1) to remove a trace of the minor diastereomer (0.38g, 0.7mmol, 45%).

m.p. 225°C

[alpha]$_D$ = -3.5° (c=2, methanol)

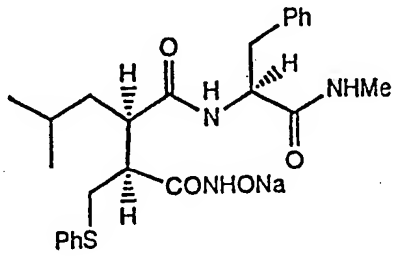
Analysis calculated for $C_{24}H_{39}N_3O_5S.0.5\ H_2O$
Requires: C58.99 H7.84 N8.60
Found: C58.96 H7.63 N8.55

delta$_H$ (250MHz, D_6-DMSO) 8.81 (1H, s, J = 1.5Hz, NHOH), 8.30 (1H, d, J=8Hz, CONH), 7.78 (1H, d, J=6Hz, CONHMe), 7.27-7.03 (5H, m, aromatic H), 4.54 (1H, m, CHCH$_2$Ph), 2.94 (1H, dd, J = 12,5Hz, CHCH$_2$Ph), 2.79 (1H, dd, J = 13,10Hz, CHCH$_2$Ph) 2.56 (3H, d, J = 4.5Hz, NHCH$_3$), 2.44 (2H, m), 2.20 (1H, dd, J = 13,3Hz, CH$_2$S), 2.07 (1H, dt), 1.36 (2H, m), 1.13 (9H, s, C(CH$_3$)$_3$), 0.87 (1H, m, CH$_2$CH(CH$_3$)$_2$), 0.79 (3H, d, J = 6Hz, CH(CH$_3$)$_2$), and 0.74 (3H, d, J = 6Hz, CH(CH$_3$)$_2$).

delta$_C$ (63.9MHz, D_6-DMSO) 172.55, 171.59, 168.24, 138.03, 129.18, 128.00, 126.24, 54.21, 46.48, 45.84, 45.55, 37.61, 28.30, 27.13, 25.64, 25.25, 24.24, and 21.63.

Example 8

[4-(N-Hydroxyamino)-2R-isobutyl-3S-(phenylthiomethyl) succinyl]-L-phenylalanine-N-methylamide sodium salt



[4-(N-Hydroxyamino)-2R-isobutyl-3S-(phenylthiomethyl) succinyl]-L-phenylalanine-N-methylamide (0,2g, 0.4 mmol) was dissolved in 20ml of methanol and 1eq of 0.1N NaOH(aq) added. The solvent was removed in vacuo and the residue dissolved in water and freeze-dried (0.21g,0.4 mmol,100%).

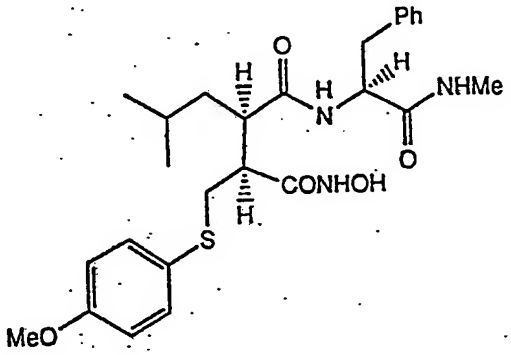
m.p. 184°C

$[\alpha]_D$ = -7.7° (c=2, methanol)

δ_H (250MHz, D_6-DMSO) 8.62 (1H, s, J = 1.5Hz, NHOH), 8.28 (1H, d, J = 8Hz, CONH), 7.26 - 7.04 (10H, m, aromatic H), 4.43 (1H, m, $CHCH_2Ph$), 3.00 (1H, dd, J = 14,4Hz, $CHCH_2Ph$), 2.84 (1H, dd, J = 14,10Hz, $CHCH_2Ph$), 2.55 (3H, d, J = 4.5Hz, $NHCH_3$), 2.46 (3H, m), 2.21 (1H, m), 1.39 (1H, m), 1.14 (1H, m), 1.00 (1H,m), and 0.70 (6H, d , J = 5.7Hz)

Example 9

[4-(N-Hydroxyamino)-2R-isobutyl-3S-(4-methoxyphenyl-thiomethyl)



succinyl]-L-phenylalanine-N-methylamide[4-Hydroxy-2R-isobutyl-3S-(4-methoxyphenylthiomethyl)succinyl]-L-phenylalanine-N-methylamide (0,5g, 1 mmol) and HOBT (0.18g, 1.2 mmol) were dissolved in 1:1 DCM/DMF and the mixture cooled to 0°C before adding WSDCI (0.23g, 1.2mmol) and NMM (0.12g, 1.2mmol). The mixture was stirred at 0°C for 1h to ensure complete formation of the activated ester. Hydroxylamine hydrochloride (0.1g, 1.5mmol) and NMM (0.15g, 1.5mmol) were dissolved in DMF then this mixture was added dropwise to the cooled solution of the activated ester. After 1h the reaction was poured into ether/water (1:1) whereupon the desired product precipitated as white crystals. These were collected by filtration, further washed with ether and water, then dried under vacuum at 50°C. This material was recrystallised from methanol/water (1:1) to remove a trace of the minor diastereomer (0.36g, 0.7mmol, 72%).

m.p. 225°C

$[\alpha]_D$ = +8° (c=0.5, methanol)

Analysis calculated for $C_{26}H_{35}N_3O_5S$
Requires: C62.25 H7.04 N8.38
Found: C62.43 H7.09 N8.37

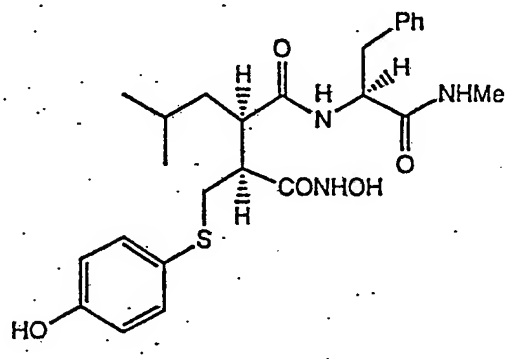
δ_H (250MHz, D_6-DMSO) 8.83 (1H, s, J = 1.5Hz, NHOH), 8.28 (1H, d, J = 8Hz, CONH), 7.83 (1H, d, J = 6Hz, CONHMe), 7.28 - 6.86 (9H, m, aromatic H), 4.52 (1H, m, $CHCH_2Ph$), 3.73 (3H, s, OCH_3), 2.91 (1H, dd, J = 14,4Hz, $CHCH_2Ph$), 2.75 (1H, dd, J = 14,10Hz, $CHCH_2Ph$), 2.57 (3H, d, J = 4.5Hz, $NHCH_3$), 2.50 - 2.34 (2H,m), 2.16 -

1.99 (2H, m, $CH_2CH(CH3)_2$) 1.36 (2H, m), 0.88 (1H, m, $C\underline{H}_2CH(CH_3)_2$), 0.80 (3H, d, J = 6Hz, $CH(C\underline{H}_3)_2$), and 0.73 (3H, d, J = 6Hz, $CH(C\underline{H}_3)_2$).

$delta_C$ (63.9MHz, D_6-DMSO) 172.79, 171.62, 168.39, 138.14, 131.34, 129.19, 128.00, 126.44, 114.59, 55.32, 54.20, 38.68, 25.63, 25.17, 24.26, and 21.70.

Example 10

[4-(N-Hydroxyamino)-2R-isobutyl-3S-(4-hydroxyphenyl-thiomethyl) succinyl]-L-phenylalanine-N-methylamide



[4-Hydroxy-2R-isobutyl-3S-(4-hydroxyphenylthiomethyl) succinyl]-L-phenylalanine-N-methylamide (0.4g, 0.8 mmol) and HOBT (0.15g, 1.0 mmol) were dissolved in 1:1 DCM/DMF and the mixture cooled to 0°C before adding WSDCI (0.20g, 1.0mmol) and NMM (0.1g, 1.0mmol). The mixture was stirred at 0°C for 1h to ensure complete formation of the activated ester. Hydroxylamine hydrochloride (0.09g, 1.3mmol) and NMM (0.13g,1.3mmol) were dissolved in DMF then this mixture was added dropwise to the cooled solution of the activated ester. After 1h the reaction was poured into ether/water (1:1)

whereupon the desired product precipitated as white crystals. These were collected by filtration, further washed with ether and water, then dried under vacuum at 50°C. This material was recrystallised from methanol/water (1:1) to remove a trace of the minor diastereomer (0.13g, 0.2mmol, 31%).

m.p. 216°C

$[\alpha]_D$ = -65° (c=0.5, methanol)

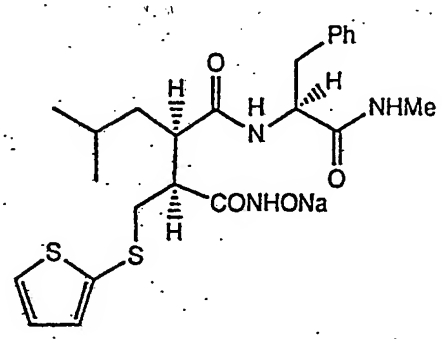
Analysis calculated for $C_{25}H_{33}N_3O_5S$
Requires: C61.58 H6.82 N8.62
Found: C61.43 H6.81 N8.08

δ_H (250MHz, D_6-DMSO) 8.82 (1H, s, J = 1.5Hz, NHOH), 8.26 (1H, d, J = 8Hz, CONH), 7.81 (1H, d, J = 6Hz, CONHMe), 7.27 - 6.64 (9H, m, aromatic H), 4.49 (1H, m, CHCH$_2$Ph), 2.90 (1H, dd, J=14,4Hz, CHCH$_2$Ph), 2.74 (1H, dd, J=14,10Hz, CHCH$_2$Ph), 2.57 (3H, d, J = 4.5Hz, NHCH$_3$), 2.54 - 2.29 (2H, m), 2.14 - 1.98 (2H, m, CH$_2$CH(CH3)$_2$), 1.35 (2H, m), 0.88 (1H, m, CH$_2$CH(CH$_3$)$_2$), 0.80 (3H, d, J = 6Hz, CH(CH$_3$)$_2$), and 0.73 (3H, d, J = 6Hz, CH(CH$_3$)$_2$).

δ_C (63.9MHz, D_6-DMSO) 172.81, 171.66, 168.46, 156.50, 133.02, 132.17, 129.17, 128.02, 126.44, 124.17, 116.00, 54.20, 46.35, 46.13, 37.59, 35.40, 25.62, 25.16, 24.27, and 21.69.

Example 11

[4-(N-Hydroxyamino)-2R-isobutyl-3S-(2-thiophenethiomethyl)succinyl]-L-phenylalanine-N-methylamide sodium salt



[4-Hydroxyamino)-2R-isobutyl-3S-(2-thiophenethiomethyl) succinyl]-L-phenylalanine-N-methylamide (0,2g, 0.4 mmol) was dissolved in 20ml of methanol and 1eq of 0.1N NaOH(aq) added. The solvent was removed in vacuo and the residue dissolved in water and freeze-dried (0.21g,0.4 mmol,100%).

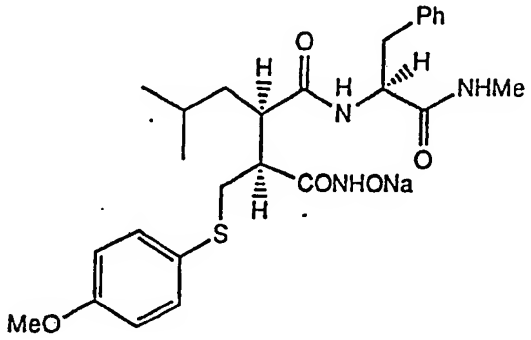
m.p. 170°C

$[\alpha]_D$ = -67° (c=1, methanol)

δ_H (250MHz, d_6-DMSO), 7.51 (1H, d), 7.19 - 6.97 (8H, m, aromatic H), 4.32 (1H, m, $C\underline{H}CH_2Ph$), 3.00 (1H, dd, J = 14,4Hz, $CHC\underline{H}_2Ph$), 2.84 (1H, dd, J = 14,10Hz, $CHC\underline{H}_2Ph$) 2.53 (3H, d, J = 4.5Hz, $NHC\underline{H}_3$), 2.46 2.19 (3H, m), 1.37 (1H, m), 1.09 (1H, m), 0.93 (1H, m), and 0.67 (6H, m)

Example 12

[4-(N-Hydroxyamino)-2R-isobutyl-3S-(4-methoxyphenyl-thiomethyl)succinyl]-L-phenylalanine-N-methylamide sodium salt



[4-Hydroxyamino)-2R-isobutyl-3S-(4-methoxyphenylthio-methyl)succinyl]-L-phenylalanine-N-methylamide (0,1g, 0.2 mmol) was dissolved in 20ml of methanol and 1eq of 0.1N NaOH(aq) added. The solvent was removed in vacuo and the residue dissolved in water and freeze-dried (0.1g,0.2 mmol,100%).

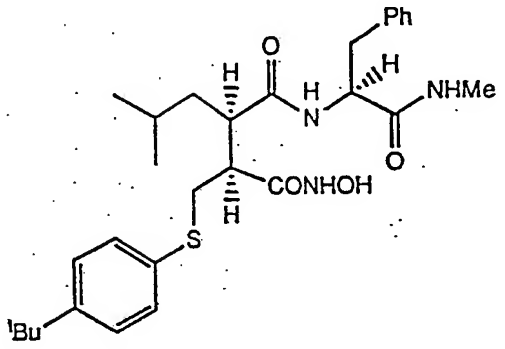
m.p. 174°C

$[\alpha]_D$ = -58° (c=1, methanol)

δ_H (250MHz, D_6-DMSO 7.26 - 7.04 (10H, m, aromatic H), 4.31 (1H, m, $C\underline{H}CH_2Ph$), 3.73 (3H, s, $OC\underline{H}_3$), 3.25 - 2.72 (2H, m, $CHCH_2Ph$), 2.50 (3H, s, $NHC\underline{H}_3$), 2.36 (1H, m), 2.15 (1H, m), 1.37 (1H, m), 0.95 (1H, m), and 0.69 (6H, d, $CHCH_2(C\underline{H}_3)_2$).

Example 13

[4-(N-Hydroxyamino)-2R-isobutyl-3S-(4-tertbutylphenyl-thiomethyl) succinyl]-L-phenylalanine-N-methylamide



[4-Hydroxy-2R-isobutyl-3S-(4-tertbutylphenylthiomethyl) succinyl]-L-phenylalanine-N-methylamide (5.0g, 10 mmol) and HOBT (1.76g, 12 mmol) were dissolved in 1:1 DCM/DMF and the mixture cooled to 0°C before adding WSDCI (2.3g, 12mmol) and NMM (1.2g, 12mmol). The mixture was stirred at 0°C for 1h to ensure complete formation of the activated ester. Hydroxylamine hydrochloride (1.0g, 15mmol) and NMM (1.2g, 15mmol) were dissolved in DMF then this mixture was added dropwise to the cooled solution of the activated ester. After 1h the reaction was poured into ether/water (1:1) whereupon the desired product precipitated as white crystals. These were collected by filtration, further washed with ether and water, then dried under vacuum at 50°C. This material was repeatedly recrystallised from methanol/water (1:1) to remove a trace of the minor diastereomer (0.7g, 1.3mmol, 14%).

M.p. 188.5 -190°C

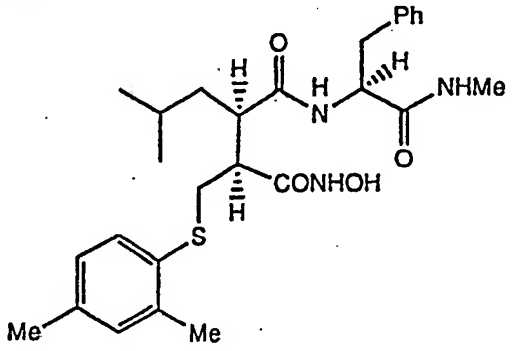
Analysis calculated for $C_{29}H_{41}N_3O_4S$
Requires: C66.00 H7.83 N7.96
Found: C65.80 H7.81 N7.76

$delta_H$ (250MHz, D_6-DMSO) 8.83 (1H, s, NHOH), 8.33 (1H, d, J = 8Hz, CONH), 7.86 (1H, d, J = 6Hz, CONHMe), 7.28 - 6.90 (9H, m, aromatic H), 4.60 (1H, m, $CHCH_2Ph$), 2.94 (1H, dd, J = 14,4Hz, $CHCH_2Ph$), 2.77 (1H, dd, J = 14,10Hz, $CHCH_2Ph$), 2.58 (3H, d, J = 4.5Hz, $NHCH_3$), 2.55 - 2.37 (2H, m), 2.22 - 2.08 (2H, m, $CH_2CH(CH3)_2$), 1.37 (2H, m), 1.26 (9H, s, $C(CH_3)_3$), 0.88 (1H, m, $CH_2CH(CH_3)_2$), 0.81 (3H, d, J = 6Hz, $CH(CH_3)_2$), and 0.74 (3H, d, J = 6Hz, $CH(CH_3)_2$).

$delta_C$ (63.9MHz, D_6-DMSO) 172.88, 171.59, 168.34, 147.87, 138.10, 133.09, 129.13, 127.95, 127.45, 126.36, 125.70, 54.19, 54.20, 46.38, 46.06, 37.70, 34.20, 32.79 31.24, 25.64, 25.19, 24.25, and 21.72.

Example 14

[4-(N-Hydroxyamino)-2R-isobutyl-3S-(2,4-dimethylphenylthiomethyl) succinyl]-L-phenylalanine-N-methylamide



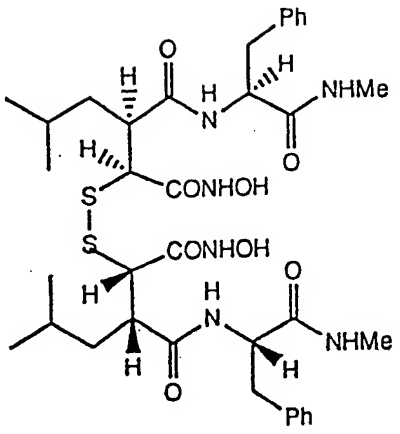
[4-Hydroxy-2R-isobutyl-3S-(2,4-dimethylphenylthiomethyl)succinyl]-L-phenylalanine-N-methylamide (1.8g, 3.7 mmol) and HOBT (0.67g, 12 mmol) were dissolved in 1:1 DCM/DMF and the mixture cooled to 0°C before adding WSDCI (0.86g, 4.5mmol) and NMM (0.45g, 4.5mmol). The mixture was stirred at 0°C for 1h to ensure complete formation of the activated ester. Hydroxylamine hydrochloride (0.39g, 5.6mmol) and NMM (0.56g, 5.6mmol) were dissolved in DMF then this mixture was added dropwise to the cooled solution of the activated ester. After 1h the reaction was poured into ether/water (1:1) whereupon the desired product precipitated as white crystals. These were collected by filtration, further washed with ether and water, then dried under vacuum at 50°C. This material was repeatedly recrystallised from methanol/water (1:1) to remove a trace of the minor diastereomer (1.08g, 2.2mmol, 58%).

m.p. 226°C (dec.)

Analysis calculated for $C_{27}H_{37}N_3O_4S$
Requires: C64.90 H7.46 N8.41
Found: C65.15 H7.48 N8.40

$delta_H$ (250MHz, D_6-DMSO) 8.83 (1H, s, NHOH), 8.32 (1H, d, J = 8Hz, CONH), 7.85 (1H, d, J = 6Hz, CONHMe), 7.30 - 6.71 (9H, m, aromatic H), 4.56 (1H, m, $CHCH_2Ph$), 2.91 (1H, dd, J = 14,4Hz, $CHCH_2Ph$), 2.76 (1H, dd, J = 14,10Hz, $CHCH_2Ph$), 2.57 (3H, d, J = 4.5Hz, $NHCH_3$), 2.53 - 2.38 (2H, m), 2.23 (3H, s, $C_6H_5(CH_3)2$), 2.13 (3H, s, $C_6H_5(CH_3)$), 1.30 (2H, m), 0.89 (1H, m, $CH_2CH(CH_3)_2$), 0.81 (3H, d, J = 6Hz, $CH(CH_3)_2$), and 0.74 (3H, d, J = 6Hz, $CH(CH_3)_2$).

Example 15

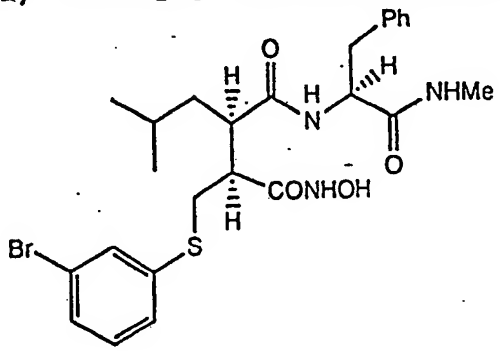


[4(N-Hydroxyamino-2R-isobutyl-3S-(acetylthiomethyl) succinyl]-L-phenylalanine-N-methylamide (1.0g, 2.4 mmol) was dissolved in 750ml methanol and 350ml pH 7 buffer added. Left to stand overnight and solvent removed in vacuo to 2/3 volume,left to crystallise for a further two hours. Filtered and dried to give 0.87g off-white crystals

Analysis calculated for $C_{38}H_{56}N_6O_8S_2.1.9H2O$
Requires: C55.34 H6.93 N9.88
Found: C55.44 H7.32 N10.21

Example 16

[4-(N-Hydroxyamino)-2R-isobutyl-3S-(3-bromophenyl-thiomethyl) succinyl]-L-phenylalanine-N-methylamide



Prepared by the method described in example 1g to give material with the following characteristics.

m.p. 225 -229°C

$[\alpha]_D = -164.8°$

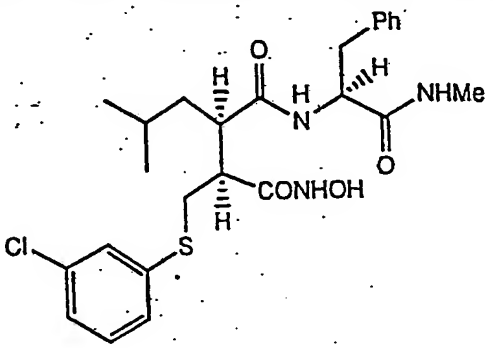
Analysis calculated for $C_{25}H_{32}BrN_3O_4S$
Requires: C54.40 H5.89 N7.40
Found: C54.54 H5.86 N7.63

δ_H (250MHz, D_6-DMSO) 8.83 (1H, s, NHOH), 8.35 (1H, d, J = 8Hz, CONH), 7.90 (1H, q, J = 6Hz, CONHMe), 7.35 - 6.87 (9H, m, aromatic H), 4.64 (1H, m, $CHCH_2Ph$), 2.94 (1H, dd, J = 14,4Hz, $CHCH_2Ph$), 2.76 (1H, t, J = 13Hz, $CHCH_2Ph$) 2.60 (3H, d, J = 5Hz, $NHCH_3$), 2.55 - 2.35 (2H, m, CH_2S), 2.15 (1H, t, J = 10Hz, CHCO), 2.01 (1H, d, J = 11.5Hz, CHCO), 1.37 (2H, m), 0.88 (1H, m, $CH_2CH(CH_3)_2$), 0.81 (3H, d, J = 6Hz, $CH(CH_3)_2$), and 0.74 (3H, d, J = 6Hz, $CH(CH_3)_2$).

δ_C (63.9MHz, D_6-DMSO) 173.0, 171.0, 168.8, 139.8, 138.0, 130.5, 129.0, 128.5, 127.5, 125.8, 125.5, 54.2, 46.0, 45.5, 38.0, 31.5, 25.5, 25.2, 24.7, and 21.0.

Example 17

[4-(N-Hydroxyamino)-2R-isobutyl-3S-(3-chlorophenylthio-methyl) succinyl]-L-phenylalanine-N-methylamide



Prepared by the method described in example 1g to give material with the following characteristics.

m.p. 231-234°C

$[\alpha]_D = -96.5°$

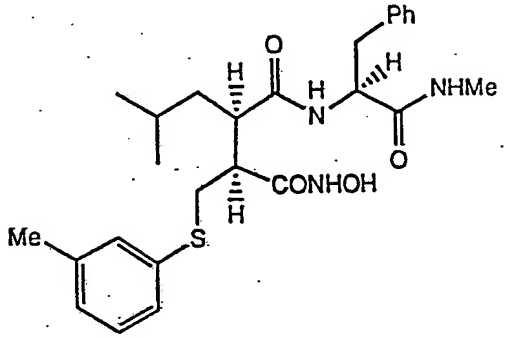
Analysis calculated for $C_{25}H_{32}ClN_3O_4S$
Requires: C59.34 H6.37 N8.30
Found: C59.51 H6.43 N8.24

δ_H (250MHz, D_6-DMSO) 8.85 (1H, s, NHOH), 8.37 (1H, d, J = 8.5Hz, CONH), 7.90 (1H, m, CONHMe), 7.30 - 6.88 (9H, m, aromatic H), 4.66 (1H, m, CHCH$_2$Ph), 2.96 (1H, bd, J = 14Hz, CHCH$_2$Ph), 2.76 (1H, bt, J = 13Hz, CHCH$_2$Ph) 2.60 (3H, d, J = 5Hz, NHCH$_3$), 2.55 - 2.40 (2H, m, CH$_2$S), 2.16 (1H, m, CHCO), 2.01 (1H, d, J = 14Hz, CHCO), 1.37 (2H, m), 0.91 (1H, m, CH$_2$CH(CH$_3$)$_2$), 0.81 (3H, d, J = 6Hz, CH(CH$_3$)$_2$), and 0.74 (3H, d, J = 6Hz,CH(CH$_3$)$_2$).

δ_C (63.9MHz, D_6-DMSO) 172.7, 171.6, 168.1, 139.2, 138.1, 130.3, 129.2, 127.9, 126.2, 125.9, 125.5, 125.0, 54.1, 46.3, 45.8, 37.8, 32.0, 25.7, 25.2, 24.2, and 21.7.

Example 18

[4-(N-Hydroxyamino)-2R-isobutyl-3S-(3-methylphenylthiomethyl) succinyl]-L-phenylalanine-N-methylamide



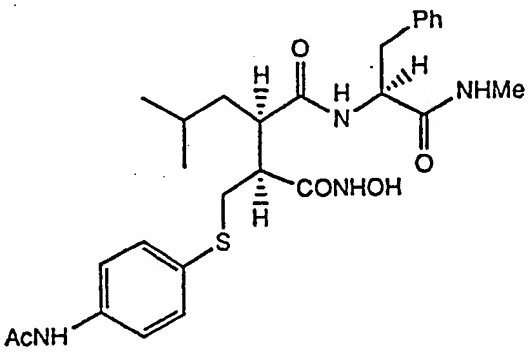
Prepared by the method described in example 1g to give material with the following characteristics.

Analysis calculated for $C_{26}H_{35}N_3O_4S$
Requires: C64.30 H7.26 N8.65
Found: C63.81 H7.21 N8.48

$delta_H$ (250MHz, D_6-DMSO) 8.83 (1H, s, NHOH), 8.35 (1H, d, J = 8.5Hz, CONH), 7.86 (1H, m, CONHMe), 7.28 - 6.77 (9H, m, aromatic H), 4.66 (1H, m, $CHCH_2Ph$), 2.96 (1H, dd, J = 14,4Hz, $CHCH_2Ph$), 2.80 (1H, bt, J = 13Hz, $CHCH_2Ph$) 2.59 (3H, d, J = 5Hz, $NHCH_3$), 2.55 - 2.37 (2H, m, CH_2S), 2.16 (2H, m, 2x$CHCO$), 1.38 (2H, m), 0.91 (1H, m, $CH_2CH(CH_3)_2$), 0.81 (3H, d, J = 6Hz, $CH(CH_3)_2$), and 0.74 (3H, d, J = 6Hz, $CH(CH_3)_2$).

Example 19

[4-(N-Hydroxyamino)-2R-isobutyl-3S-(4-(N-acetyl)-aminophenylthiomethyl)succinyl]-L-phenylalanine-N-methylamide.



A) [2R-isobutyl-3S-(4-aminophenylthiomethyl)succinyl]-L-phenylalanine -N-methylamide.

Prepared by the method described in example 1f to give material with the following characteristics.

$delta_H$ (250MHz, D_6-DMSO) 8.27 (1H, d, J = 8.5Hz, CONH), 7.81 (1H, m, CONHMe), 7.30 - 7.00 (5H, m, phenyl H), 6.86 (2H, d, J = 8.5Hz, aromatic H), 6.45 (2H, d, J = 8.5Hz, aromatic H), 5.25 (1H, bs, CO_2H), 4.48 (1H, m, $CHCH_2Ph$), 2.91 (1H, dd, J = 14,4Hz, $CHCH_2Ph$), 2.88 (1H, dd, J = 14,10Hz, $CHCH_2Ph$) 2.56 (3H, d, J = 5Hz, $NHCH_3$), 2.43 - 2.24 (3H, m, CH_2S and CHCO), 2.03 (1H, d, J = 10Hz, CHCO), 1.41 (1H, t, J = 11Hz, $CH_2CH(CH_3)_2$), 1.26 (1H, m, $CH_2CH(CH_3)_2$), 0.85 (1H, m, $CH_2CH(CH_3)_2$), 0.81 (3H, d, J = 6Hz, $CH(CH_3)_2$), and 0.74 (3H, d, J=6Hz, $CH(CH_3)_2$).

B) [2R-isobutyl-3S-(4-(N-acetyl)aminophenyl-thiomethyl)- succinyl]-Lphenylalanine-N-methylamide.

The product from above (350mg, 0.74 mmol) was dissolved in DCM (5 ml) cooled in an ice bath then triethylamine (75mg, 0.74 mmol), DMAP (91mg, 7.4 mmol) and finally acetic anhydride (83mg, 8.2 mmol) were added and the solution stirred at RT for 90 minutes. The mixture was partitioned between ethyl acetate and citric acid then the organic layer washed with water and finally dried over magnesium sulphate. Solvent removal gave the crude product as pale yellow crystals (160mg, 0.31 mmol, 42%).

$delta_H$ (250MHz, D_6-DMSO) 9.94 (1H, s, $CO_2\underline{H}$), 8.34 (1H, d, J = 8.5Hz, CON$\underline{H}$), 7.90 (1H, m, CON$\underline{H}$Me), 7.46 (2H, d, J = 8.5Hz, aromatic H) 7.30 - 7.00 (5H, m, phenyl H), 6.96 (2H, d, J = 8.5Hz, aromatic H), 4.57 (1H, m, C$\underline{H}$CH$_2$Ph), 2.91 (1H, dd, J = 14,4Hz, CHC$\underline{H}_2$Ph), 2.88 (1H, bt, J = 13Hz, CHC$\underline{H}_2$Ph), 2.58 (3H, d, J = 5Hz, NHC$\underline{H}_3$), 2.43 - 2.16 (3H, m, C$\underline{H}_2$S and C$\underline{H}$CO), 2.10 (1H, d, J = 14Hz, C$\underline{H}$CO), 1.35 (1H, t, J = 14Hz, C$\underline{H}_2$CH(CH$_3$)$_2$), 1.26 (1H, m, CH$_2$C$\underline{H}$(CH$_3$)$_2$), 0.86 (1H, m, C$\underline{H}_2$CH(CH$_3$)$_2$), 0.81 (3H, d, J = 6Hz, CH(C$\underline{H}_3$)2), and 0.74 (3H, d, J = 6Hz,CH(C$\underline{H}_3$)$_2$).

C) [4-(N-Hydroxyamino)-2R-isobutyl-3S-(4-(N-acetyl)-aminophenylthiomethyl)succinyl]-L-phenylalanine-N-methylamide.

Prepared by the method described in example 1g to give material with the following characteristics.

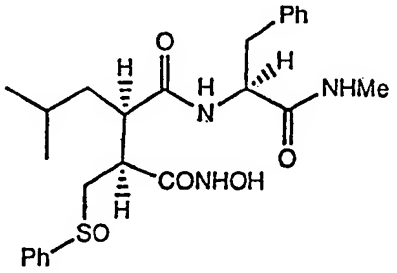
m.p. 201 -202°C (dec.)

$[\alpha]_D$ = -7.5° (c=1.0, methanol)

δ_H (250MHz, D_6-DMSO) 9.90 (1H, s, NHOH), 8.82 (1H, s, NHOH), 8.30 (1H, d, J = 8.5Hz, CONH), 7.85 (1H, m, CONHMe), 7.45 (2H, d, J = 8.5Hz, aromatic H), 7.28 - 6.94 (5H, m, phenyl H), 6.90 (2H, d, J = 8.5Hz, aromatic H), 4.66 (1H, m, $CHCH_2Ph$), 2.90 (1H, dd, J = 14,4Hz, $CHCH_2Ph$), 2.76 (1H, bt, J = 13Hz, $CHCH_2Ph$), 2.50 (3H, d, J = 5Hz, $NHCH_3$), 2.49 - 2.35 (2H, m, CH_2S), 2.14 (1H, m, CHCO), 2.03 (4H, s + m, $COCH_3$ and CHCO), 1.35 (2H, m), 0.86 (1H, m, $CH_2CH(CH_3)_2$), 0.81 (3H, d, J = 6Hz, $CH(CH_3)_2$), and 0.74 (3H, d, J = 6Hz, $CH(CH_3)_2$).

Example 20

[4-(N-Hydroxyamino)-2R-isobutyl-3S-phenylsulfinyl-methylsuccinyl]-L-phenylalanine-N-methylamide.



[4-(N-Hydroxyamino)-2R-isobutyl-3S-phenylthiomethyl-succinyl]-L-phenylalanine-N-methylamide (250mg, 0.53mmol) was dissolved in methanol (50 ml) and meta-

chloroperbenzoic acid (100mg, 0.58 mmol) was added. After stirring for 1h at room temperature ether was added and the mixture filtered. Solvent removal gave the crude white solid which was recrystallised from methanol / water then slurried in ether to remove final traces of meta-chlorobenzoic acid to give the desired material (70 mg, 0.014 mmol, 27%).

m.p. 186 -188°C

$[alpha]_D$ = -13.6° (c=0.5, methanol)

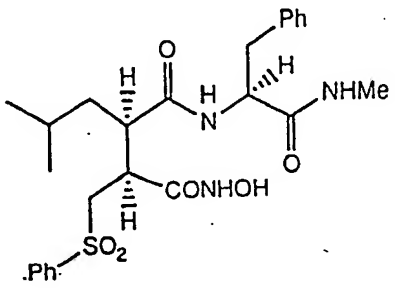
Analysis calculated for $C_{25}H_{33}N_3O_5S.0.5H_2O$
Requires: C60.46 H6.90 N8.46
Found: C60.58 H6.69 N8.29

$delta_H$ (250MHz, D_6-DMSO, mixture of diastereomers) 9.04 + 8.93 (1H, 2xs, NHOH), 8.29 + 8.16 (1H, 2xd, J = 8.5 Hz, CONH), 7.79 (1H, m, CONHMe), 7.90 - 7.40 (8H, m, aromatic H), 7.06 + 6.82 (2H, 2xm, SO-Aromatic), 4.37 (1H, m, $CHCH_2Ph$), 2.93 - 2.58 (3H, m, containing $CHCH_2Ph$), 2.52 (3H, m, $NHCH_3$), 2.49 + 2.37 (1H, 2xm), 1.49 - 1.25 (2H, m, $CH_2CH(CH_3)_2$ and $CH2CH(CH_3)_2$), 0.95 (1H, m, $CH_2CH(CH_3)_2$), 0.81 (3H, d, J = 6Hz, $CH(CH_3)_2$), and 0.74 (3H, d, J=6Hz, $CH(CH_3)_2$).

$delta_C$ (63.9MHz, D_6-DMSO, mixture of diastereomers) 172.2, 171.4, 171.3, 167.7, 144.5, 138.0, 137.9, 131.3, 130.9, 129.6, 129.3, 129.1, 128.8, 128.3, 127.8, 126.5, 126.2, 124.3, 123.6, 59.8, 58.1, 54.3, 54.0, 46.2, 45.8, 41.6, 40.9, 37.6, 37.4, 25.6, 25.0, 24.3, 24.2, 21.7, and 21.6.

Example 21

[4-(N-Hydroxyamino)-2R-isobutyl-3S-phenylsulfonyl-methylsuccinyl]-L-phenylalanine-N-methylamide.



[4-(N-Hydroxyamino)-2R-isobutyl-3S-phenylthiomethyl-succinyl]-L-phenylalanine-N-methylamide (50mg, 0.11mmol) was dissolved in methanol (12 ml) and meta-chloroperbenzoic acid (40mg, 0.23 mmol) was added. After stirring for 3h at room temperature ether was added and the mixture filtered. Solvent removal gave the crude white solid which was slurried in ether to remove final traces of meta-chlorobenzoic acid to give the desired material.

m.p. 228 - 231°C

$[\alpha]_D$ = 16.8° (c=0.5, methanol)

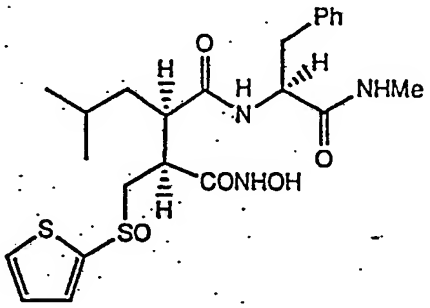
Analysis calculated for $C_{25}H_{33}N_3O_6S.0.3H_2O$
Requires: C58.99 H6.65 N8.25
Found: C58.92 H6.51 N8.05

δ_H (250MHz, D_6-DMSO) 8.66 (1H, s, NHOH), 8.25 (1H, d, J = 8.5 Hz, CONH), 7.83 (1H, m, CONHMe), 7.75 - 7.50 (5H, m, aromatic H), 7.30 7.05 (5H, m, aromatic H),

4.36 (1H, m, C<u>H</u>CH$_2$Ph), 2.86 (1H, dd, J = 14,5 Hz, CHC<u>H</u>$_2$Ph), 2.75 (1H, dd, J = 14,10 Hz, CHC<u>H</u>$_2$Ph), 2.54 (3H, d, J = 4.5 Hz, NHC<u>H</u>$_3$), 2.54 (2H, m), 1.30 (2H, m, C<u>H</u>$_2$CH(CH$_3$)$_2$ and CH$_2$C<u>H</u>(CH$_3$)$_2$), 0.86 (1H, m, C<u>H</u>$_2$CH(CH$_3$)$_2$), 0.75 (3H, d, J = 6Hz, CH(C<u>H</u>$_3$)$_2$), and 0.71 (3H, d, J = 6Hz,CH(C<u>H</u>$_3$)$_2$).

<u>Example 22</u>

[4-(N-Hydroxyamino)-2R-isobutyl-3S-thiophenylsulphinylmethyl-succinyl] -L-phenylalanine-N-methylamide



[4-(N-Hydroxyamino)-2R-isobutyl-3S-thiophenylthio-methyl-succinyl]-L-phenylalanine-N-methylamide (50mg, 0.11mmol) was treated as described in example 21 to yield the title compound (16mg, 0.03 mmol, 29%) as a mixture of diastereomer with the following characteristics:

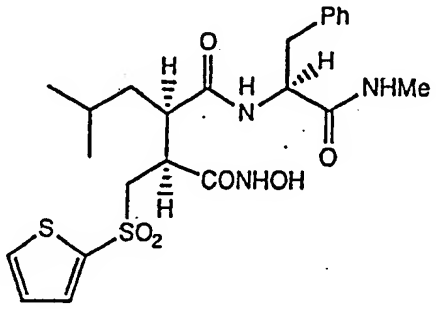
m.p. 195 -197°C (dec.)

Analysis calculated for $C_{23}H_{31}N_3O_5S_2.0.5H_2O$
Requires: C54.96 H6.42 N8.36
Found: C54.91 H6.23 N8.23

delta$_H$ (250MHz, D_6-DMSO, mixture of diastereomers) 9.04 + 8.96 (1H, 2xs, NHOH), 8.34 + 8.29 (1H, 2xd, J = 8.5 Hz, CONH), 8.02 + 7.98 (1H, 2xm, CONHMe), 7.81 (1H, bs, thiophene-H), 7.42 (1H, s, thiophene-H), 7.25 - 7.15 (5H, m, phenyl), 7.03 (1H, bs, thiophene-H), 4.43 (1H, m, $CHCH_2Ph$), 3.0 - 2.6 (4H, m, containing $CHCH_2Ph$), 2.52 (7H, m, containing $NHCH_3$), 2.05 (1H, m), 1.6 - 1.2 (2H, m, $CH_2CH(CH_3)_2$ and $CH_2CH(CH_3)_2$), 0.87 (1H, m, $CH_2CH(CH_3)_2$), and 0.85 - 0.71 (6H, m, $CH(CH_3)_2$).

Example 23

[4-(N-Hydroxyamino)-2R-isobutyl-3S-thiophenylsulphonylmethyl-succinyl] -L-phenylalanine-N-methylamide.



[4-(N-Hydroxyamino)-2R-isobutyl-3S-thiophenylthiomethyl-succinyl]-L-phenylalanine-N-methylamide (75mg, 0.16mmol) was treated as described in example 22 to yield the title compound (40mg, 0.08 mmol, 49%) with the following characteristics:

m.p. 215 - 216°C

Analysis calculated for $C_{23}H_{31}N_3O_6S_2$

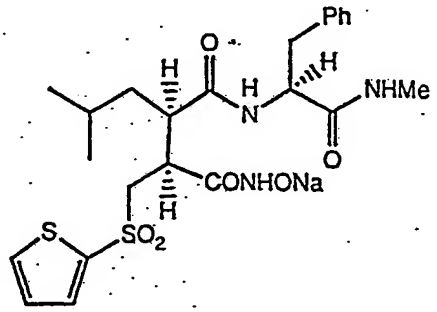
Requires: C54.21 H6.13 N8.24
Found: C54.07 H6.19 N8.04

$delta_H$ (250MHz, D_6-DMSO) 887 (1H, s, NHOH), 8.25 (1H, d, J = 8.5 Hz, CONH), 8.09 (1H, d, J = 4.7 Hz, thiophene-H), 7.83 (1H, m, CONHMe), 7.53 (1H, d, J = 3 Hz, thiophene H), 7.25 - 7.12 (6H, m, phenyl and thiophene-H), 4.36 (1H, m, $CHCH_2Ph$), 3.38 (1H, dd, J = 14,11 Hz, SCH_2), 2.87 (1H, dd, J = 14,5 Hz, $CHCH_2Ph$), 2.75 (1H, dd, J = 14,10 Hz, $CHCH_2Ph$), 2.70 - 2.36 (6H, m, containing $NHCH_3$), 1.20 (2H, m, $CH_2CH(CH_3)_2$ and $CH_2CH(CH_3)_2$), 0.89 (1H,m, $CH_2CH(CH_3)_2$), and 0.75 (6H, m, $CH(CH_3)_2$).

$delta_C$ (63.9MHz, D_6-DMSO) 172.0, 171.2, 166.5, 140.0, 138.0, 135.4, 134.6, 129.0, 128.4, 128.2, 126.6, 54.3, 45.6, 37.5, 25.6, 25.0, 24.2, and 21.7.

Example 24

[4-(N-Hydroxyamino)-2R-isobutyl-3S-phenylsulfonyl-methylsuccinyl]-L-phenylalanine-N-methylamide sodium salt.



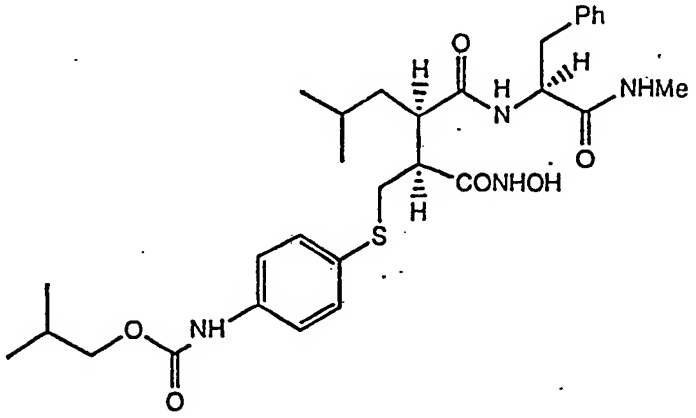
[4-(N-Hydroxyamino)-2R-isobutyl-3S-phenylsulfonyl-

methylsuccinyl]-L-phenylalanine-N-methylamide (50mg, 0.1mmol) was dissolved in methanol (10ml) and sodium hydroxide solution (0.1M, 1.0ml) added to give a homogeneous solution. The methanol was removed under reduced pressure then the residual aqueous solution freeze dried to give the title compound (40mg).

$delta_H$ (250MHz, D_6-DMSO) 8.66 (1H, s, NHOH), 8.25 (1H, d, J = 8.5 Hz, CONH), 7.83 (1H, m, CONHMe), 7.75 - 7.50 (5H, m, aromatic H), 7.30 7.05 (5H, m, aromatic H), 4.36 (1H, m, $CHCH_2Ph$), 2.86 (1H, dd, J = 14,5 Hz, $CHCH_2Ph$), 2.75 (1H, dd, J = 14,10 Hz, $CHCH_2Ph$), 2.54 (3H, d, J=4.5 Hz, $NHCH_3$), 2.54 (2H, m), 1.30 (2H, m, $CH_2CH(CH_3)_2$ and $CH_2CH(CH_3)_2$), 0.86 (1H, m, $CH_2CH(CH_3)_2$), 0.75 (3H, d, J = 6Hz, $CH(CH_3)_2$), and 0.71 (3H, d, J = 6Hz, $CH(CH_3)_2$).

Example 25

[4-(N-Hydroxyamino)-2R-isobutyl-3S-(4-(isobutyloxy-carbonylamino)phenyl)thiomethyl-succinyl]-L-phenyl-alanine-N-methylamide



a) [4-Hydroxy-2R-isobutyl-3S-(4-aminophenyl)thio-

methylsuccinyl]-L-phenylalanine-N-methylamide was prepared by the method described in example 1f to give a compound with the following characteristics.

delta$_H$ (250MHz, D_6-DMSO) 8.26 (1H, d, J = 8.5 Hz, CONH), 7.81 (1H, m, CONHMe), 7.27 - 7.15 (5H, m, phenyl H), 6.85 (2H, d, J = 8.5Hz, aromatic H), 6.46 (2H, d, J = 8.5Hz, aromatic H), 5.2 (1H, bs, CO_2H), 4.48 (1H, m, $CHCH_2Ph$), 2.90 (1H, dd, J = 13.5, 4.3 Hz, $CHCH_2Ph$), 2.75 (1H, dd, J = 13.6, 10 Hz, $CHCH_2Ph$), 2.56 (3H, d, J = 4.5 Hz, $NHCH_3$), 2.50 - 2.25 (3H, m), 2.03 (1H, d, J = 10 Hz), 1.41 (1H, m, $CH_2CH(CH_3)_2$), 1.26 (1H, m, $CH_2CH(CH_3)_2$), 0.86 (1H, m, $CH_2CH(CH_3)_2$), 0.75 (3H, d, J = 6Hz, $CH(CH_3)_2$), and 0.71 (3H, d, J = 6Hz, $CH(CH_3)_2$).

b) N,N-Dimethylglycine (100mg, 0.97 mmol) was stirred in dry THF (50ml) and triethylamine (108mg, 1.1mmol) and isobutylchloroformate (146mg, 1.1mmol) were added. After 1h the product from example 26a (500mg, 1.1mmol) was addedand the mixture stirred for a further 1h. The reaction was worked up by partitioning between citric acid and ethyl acetate, drying the organic layer and solvent removal to give the crude product (1g). Solution of the crude solid in ethyl acetate then precipitation with ether resulted in white crystals of the isobutylchloroformate derivative.

c) [4-(N-Hydroxyamino)-2R-isobutyl-3S-(4-(isobutyloxy-carbonylamino) phenyl)thiomethyl-succinyl]-L-phenyl-alanine-N-methylamide

The product from example 26b was converted to the hydroxamic acid as described in example 1g. to give a compound with the following characteristics.

m.p. 198 - 200°C

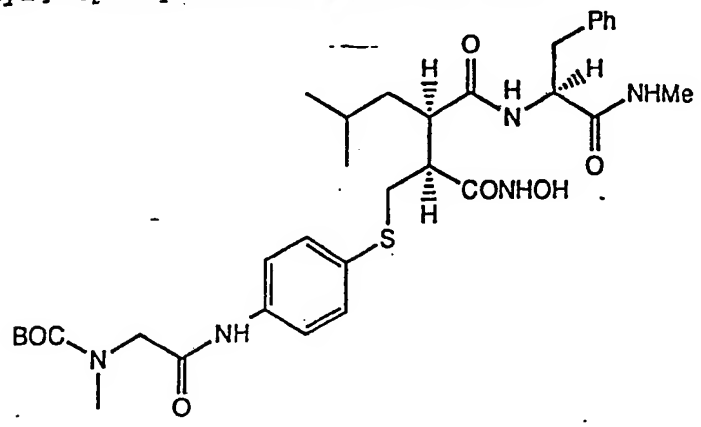
$[\alpha]_D$ = -8.5° (c=1, methanol)

Analysis calculated for $C_{30}H_{42}N_4O_6S$
Requires: C61.41 H7.22 N9.55
Found: C62.04 H7.32 N9.67

δ_H (250MHz, D_6-DMSO) 9.60 (1H, s, NHOH), 8.83 (1H, s, NHOH), 8.31 (1H, d, J = 8.5 Hz, CONH), 7.85 (1H, m, CONHMe), 7.36 - 7.25 (4H, m, aromatic H), 7.14 - 7.05 (3H, m, aromatic H), 6.91 (2H, d, J = 8.5Hz, aromatic H), 4.56 (1H, m, $CHCH_2Ph$), 3.87 (2H, d, J = 7Hz, $OCH_2CH(CH_3)_2$), 2.92 (1H, dd, J = 13.7,4.0 Hz, $CHCH_2Ph$), 2.76 (1H, dd, J = 13.6,10 Hz, $CHCH_2Ph$), 2.58 (3H, d, J = 4.5 Hz, $NHCH_3$), 2.50 - 2.34 (2H, m), 2.16 - 1.87 (3H, m), 1.35 (2H, m, $CH_2CH(CH_3)_2$ and $CH_2CH(CH_3)_2$), 0.93 (6H, d, J = 6.6Hz, $OCH_2CH(CH_3)_2$), 0.87 (1H,m, $CH_2CH(CH_3)_2$), 0.75 (3H, d, J = 6Hz, $CH(CH_3)_2$), and 0.71 (3H, d, J = 6Hz, $CH(CH_3)_2$).

Example 26

[4-(N-Hydroxyamino)-2R-isobutyl-3S-(4-(N-methyl-N-(tertbutoxycarbonyl)-glycylamino) phenyl)thiomethyl-succinyl]-Lphenylalanine-N-methylamide.



a) [4-Hydroxy-2R-isobutyl-3S-(4-(N-methyl-N-(tert-butoxycarbonyl)glycylamino) phenyl)thiomethyl-succinyl]-L-phenylalanine-N-methylamide was prepared as described in example 26b by substitution of N-BOC sarcosine for the acid component.

$delta_H$ (250MHz, D_6-DMSO) 9.97 (1H, s, $CO_2\underline{H}$), 8.36 (1H, d, J = 8.5 Hz, CONH), 7.91 (1H, m, CONHMe), 7.48 (2H, d, J = 8.5Hz, aromatic H), 7.40 - 7.05 (5H, m, aromatic H), 6.97 (2H, d, J = 8.5Hz, aromatic H), 4.58 (1H, m, $C\underline{H}CH_2Ph$), 3.95 (2H, d, J = 9Hz, $NC\underline{H}_2CO$), 2.92 (4H, m+d, $CHC\underline{H}_2Ph$ and $BOCNC\underline{H}_3$), 2.76 (1H, dd, J = 13,10 Hz, $CHC\underline{H}_2Ph$), 2.58 (3H, d, J = 4.5 Hz, $NHC\underline{H}_3$), 2.50 - 2.09 (4H, m), 1.46 - 1.33 (11H, m + 2xs, $(C\underline{H}_3)_3C$, $C\underline{H}_2CH(CH_3)_2$ and $CH_2C\underline{H}(CH_3)_2$), 0.87 (1H, m, $C\underline{H}_2CH(CH_3)_2$), 0.75 (3H, d, J = 6Hz, $CH(C\underline{H}_3)_2$), and 0.71 (3H, d, J = 6Hz, $CH(C\underline{H}_3)_2$).

b) [4-(N-Hydroxyamino)-2R-isobutyl-3S-(4-(N-methyl- N-(tertbutoxycarbonyl)-glycylamino)phenyl)- thiomethyl-succinyl]-Lphenylalanine-N-methylamide was prepared from the material produced in example 27a as described in example 1g.

$delta_H$ (250MHz, D_6-DMSO) 9.97 (1H, s, CONHOH), 8.83 (1H, s, NHOH), 8.32 (1H, d, J = 8.5 Hz, CONH), 7.86 (1H, m, CONHMe), 7.46 (2H, d, J = 8.5Hz, aromatic H), 7.28 - 7.00 (5H, m, aromatic H), 6.97 (2H, d, J = 8.5Hz, aromatic H), 4.56 (1H, m, $C\underline{H}CH_2Ph$), 3.94 (2H, d, J = 9Hz, $NC\underline{H}_2CO$), 2.87 (4H, m+d, $CHC\underline{H}_2Ph$ and $BOCNC\underline{H}_3$), 2.76 (1H, m, $CHC\underline{H}_2Ph$), 2.57 (3H, d, J = 4.5 Hz, $NHC\underline{H}_3$), 2.25 - 1.91 (2H, m), 1.42 - 1.30 (11H, m + 2xs, $(C\underline{H}_3)_3C$, $C\underline{H}_2CH(CH_3)_2$ and $CH_2C\underline{H}(CH_3)_2$), 0.92 (1H, m, $C\underline{H}_2CH(CH_3)_2$), 0.80 (3H, d, J = 6Hz, $CH(C\underline{H}_3)_2$), and 0.73 (3H, d, J=6Hz, $CH(C\underline{H}_3)_2$).

Example 27

Collagenase inhibition activity

The potency of compounds of general formula I to act as inhibitors of collagenase (a metalloproteas involved in tissue degradation) was determined by the procedure of Cawston and Barrett, (Anal. Biochem., 99, 340-345, 1979), hereby incorporated by reference, whereby a 1mM solution of the inhibitor being tested or dilutions thereof was incubated at 37° for 16 hours with collagen and collagenase (buffered with 25mM Hepes, pH 7.5 containing 5mM $CaCl_2$, 0.05% Brij 35 and 0.02% NaN_3). The collagen was acetylated ^{14}C collagen prepared by the method of Cawston and Murphy (Methods in Enzymology, 80, 711, 1981), hereby incorporated by reference. The samples were centrifuged to sediment undigested collagen and an aliquot of the radioactive supernatant removed for assay on a scintillation counter as a measure of hydrolysis. The collagenase activity in the presence of 1 mM inhibitor, or a dilution thereof, was compared to activity in a control devoid of inhibitor and the results reported below as that inhibitor concentration effecting 50% inhibition of the collagenase (IC_{50}).

Compound of Example No.	IC_{50}
1	20 nM
2	8 nM
5	3 nM
6	(50% @ 1 mcM)

Example 28

Stromelysin inhibition activity

The potency of compounds of general formula I to act as inhibitors of stromelysin was determined using the procedure of Cawston et al (Biochem. J., 195, 159-165 1981), hereby incorporated by reference, whereby a 1mM solution of the inhibitor being tested or dilutions thereof was incubated at 37°C for 16 hours with stromelysin and ^{14}C acetylate casein (buffered with 25mM Hepes, pH 7.5 containing 5mM $CaCl_2$, 0.05% Brij 35 and 0.02% NaN_3. The casein was ^{14}C acetylated according to the method described in Cawston et al (Biochem. J., 195, 159-165, 1981), hereby incorporated by reference. The stromelysin activity in the presence of 1mM, or a dilution thereof, was composed to activity in a control devoid of inhibitor and the results reported below as that inhibitor concentration effecting 50% inhibition of the stromelysin (IC_{50}).

Compound of Example No.	IC_{50}
1	10 nM
2	20 nM

Examples of unit dosage compositions are as follows:

Example 29

Capsules:

Ingredients	Per Capsule	Per 10,000 Capsules
1. Active ingredient Cpd. of Form. I	40.0 mg	400 g
2. Lactose	150.0 mg	1500 g
3. Magnesium stearate	4.0 mg	40 g
	194.0 mg	1940 g

Procedure for capsules:

Step 1. Blend ingredients No. 1 and No. 2 in a suitable blender.

Step 2. Pass blend from Step 1 through a No. 30 mesh (0.59 mm) screen.

Step 3. Place screened blend from Step 2 in a suitable blender with ingredient No. 3 and blend until the mixture is lubricated.

Step 4. Fill into No. 1 hard gelatin capsule shells on a capsule machine.

Example 30

Tablets:

	Ingredients	Per Tablet	Per 10,000 Tablets
1.	Active ingredient Cpd. of Form. I	40.0 mg	400 g
2.	Corn Starch	20.0 mg	200 g
3.	Alginic acid	20.0 mg	200 g
4.	Sodium alginate	20.0 mg	200 g
5.	Magnesium stearate	1.3 mg	13 g
		101.3 mg	1013 g

Procedure for tablets:

Step 1. Blend ingredients No. 1, No. 2, No. 3 and No. 4 in a suitable mixer/blender.

Step 2. Add sufficient water portionwise to the blend from Step 1 with careful mixing after each addition. Such additions of water and mixing until the mass is of a consistency to permit its conversion to wet granules.

Step 3. The wet mass is converted to granules by passing it through an oscillating granulator using a No. 8 mesh (2.38) screen.

Step 4. The wet granules are then dried in an oven at 140°F (60°C) until dry.

Step 5. The dry granules are lubricated with ingredient No. 5.

Step 6. The lubricated granules are compressed on a suitable tablet press.

Example 31

Intramuscular Injection:

	Ingredient	Per ml.	Per liter
1.	Compound of Formula I Active ingredient	10.0 mg	10 g
2.	Istonic buffer solution pH 4.0.	q.s.	q.s.

Procedure:

Step 1. Dissolve the active ingredient in the buffer solution.

Step 2. Aseptically filter the solution from Step 1.

Step 3. The sterile solution is now aseptically filled into sterile ampoules.

Step 4. The ampoules are sealed under aspetic conditions.

Example 32

Suppositories:

	Ingredients	Per Supp.	Per 1,000 Supp
1.	Compound of Form. I Active ingredient	40.0 mg	40 g
2.	Polyethylene Glycol 1000	1350.0 mg	1,350 g
3.	Polyethylene Glycol 4000	450.0 mg	450 g
		1840.0 mg	1,840 g

Procedure:

Step 1. Melt ingredient No. 2 and No. 3 together and stir until uniform.

Step 2. Dissolve ingredient No. 1 in the molten mass from Step 1 and stir until uniform.

Step 3. Pour the molten mass from Step 2 into suppository moulds and chill.

Step 4. Remove the suppositories from moulds and wrap.

Example 33

Eye Ointment

An appropriate amount of a compound of general formula I is formulated into an eye ointment base having the following composition:

Liquid paraffin	10%
Wool fat	10%
Yellow soft paraffin	80%

Example 34

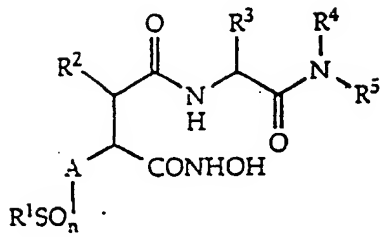
Topical skin ointment

An appropriate amount of a compound of general formula I is formulated into a topical skin ointment base having the following composition:

Emulsifying wax	30%
White soft paraffin	50%
Liquid paraffin	20%

CLAIMS

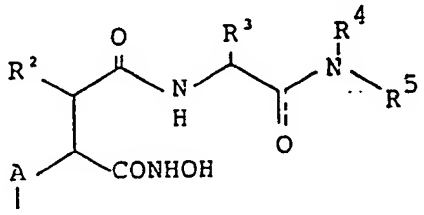
1. A compound of general formula I:



(I)

wherein:

R^1 represents a C_1-C_6 alkyl, phenyl, thiophenyl, substituted phenyl, phenyl(C_1-C_6)alkyl, heterocyclyl, (C_1-C_6)alkylcarbonyl or phenacyl or substituted phenacyl group; or when n = 0, R^1 represents SR^X, wherein R^X represents a group:



;

R^2 represents a hydrogen atom or a C_1-C_6 alkyl, C_1-C_6 alkenyl, phenyl(C_1-C_6)alkyl, cycloalkyl(C_1-C_6)alkyl or cycloalkenyl(C_1-C_6)alkyl group;

R^3 represents an amino acid side chain or a C_1-C_6 alkyl, benzyl, (C_1-C_6 alkoxy)benzyl or benzyloxy(C_1-C_6 alkyl) or benzyloxy benzyl group;

R^4 represents a hydrogen atom or a C_1-C_6 alkyl group;

R^5 represents a hydrogen atom or a methyl group;

n is an integer having the value 0, 1 or 2; and

A represents a C_1-C_6 hydrocarbon chain, optionaly substituted with one or more C_1-C_6 alkyl, phenyl or substituted phenyl groups;

or a salt thereof.

2. A compound as claimed in Claim 1, in which the chiral centre adjacent the substituent R^3 has S stereochemistry.

3. A compound as claimed in Claim 1 or 2, wherein the chiral centre adjacent the substituent R^2 has R stereochemistry.

4. A compound as claimed in Claim 1, 2 or 3, in which R^1 represents a hydrogen atom or a C_1-C_4 alkyl, phenyl, thiophenyl, benzyl, acetyl or phenacyl group.

5. A compound as claimed in any one of Claims 1 to 4, wherein R^2 represents a C_3-C_6 alkyl group.

6. A compound as claimed in any one of Claims 1 to 5, wherein R^3 represents a benzyl or 4-(C_1-C_6)alkoxyphenylmethyl or benzyloxybenzyl group.

7. A compound as claimed in any one of Claims 1 to 6, wherein R^4 represents a C_1-C_4 alkyl group.

8. A compound as claimed in any one of Claims 1 to 7, wherein R^5 represents a hydrogen atom.

9. [4-(N-Hydroxyamino)-2R-isobutyl-3S-(phenylthiomethyl)-succinyl]-L-phenylalanine-N-methylamide,

[4-(N-Hydroxyamino)-2R-isobutyl-3S-(thiophenylthiomethyl) succinyl]-L-phenylalanine-N-methylamide,

[4-(N-Hydroxyamino)-2R-isobutyl-3S-(benzylthiomethyl) succinyl]-L-phenylalanine-N-methylamide,

[4-(N-Hydroxyamino)-2R-isobutyl-3S-(acetylthiomethyl) succinyl]-L-phenylalanine-N-methylamide or

[4-(N-Hydroxyamino)-2R-isobutyl-3S-(thiolmethyl) succinyl]-L-phenylalanine-N-methylamide

[4-(N-Hydroxyamino)-2R-isobutyl-3S-(pivaloylthiomethyl) succinyl]-L-phenylalanine-N-methylamide

[4-(N-Hydroxyamino)-2R-isobutyl-3S-(phenylthiomethyl) succinyl]-L-phenylalanine-N-methylamide sodium salt

[4-(N-Hydroxyamino)-2R-isobutyl-3S-(4-methoxyphenylthiomethyl)succinyl]-L-phenylalanine-N-methylamide

[4-(N-Hydroxyamino)-2R-isobutyl-3S-(4-hydroxyphenylthiomethyl)succinyl]-L-phenylalanine-N-methylamide

[4-(N-Hydroxyamino)-2R-isobutyl-3S-(2-thiophenethiomethyl)succinyl]-L-phenylalanine-N-methylamide sodium salt

[4-(N-Hydroxyamino)-2R-isobutyl-3S-(4-methoxyphenyl-thiomethyl)succinyl]-L-phenylalanine-N-methylamide sodium salt

[4-(N-Hydroxyamino)-2R-isobutyl-3S-(4-tertbutylphenyl-thiomethyl)succinyl]-L-phenylalanine-N-methylamide

[4-(N-Hydroxyamino)-2R-isobutyl-3S-(2,4-dimethylphenyl-thiomethyl) succinyl]-L-phenylalanine-N-methylamide

bis-S,S'-([4(N-Hydroxyamino-2R-isobutyl-3S-(thiomethyl) succinyl]-L-phenylalanine-N-methylamide) disulphide

[4-(N-Hydroxyamino)-2R-isobutyl-3S-(3-bromophenylthio-methyl) succinyl]-L-phenylalanine-N-methylamide

[4-(N-Hydroxyamino)-2R-isobutyl-3S-(3-chlorophenylthio-methyl) succinyl]-L-phenylalanine-N-methylamide

[4-(N-Hydroxyamino)-2R-isobutyl-3S-(3-methylphenylthio-methyl) succinyl]-L-phenylalanine-N-methylamide

[4-(N-Hydroxyamino)-2R-isobutyl-3S-(4-(N-acetyl)-amino-phenylthiomethyl)succinyl]-L-phenylalanine-N-methyl-amide

[4-(N-Hydroxyamino)-2R-isobutyl-3S-phenylsulphinyl-methylsuccinyl]-L-phenylalanine-N-methylamide

[4-(N-Hydroxyamino)-2R-isobutyl-3S-phenylsulphonyl-methylsuccinyl]-L-phenylalanine-N-methylamide

[4-(N-Hydroxyamino)-2R-isobutyl-3S-thiophenylsulphinyl-methyl-succinyl]-L-phenylalanine-N-methylamide

[4-(N-Hydroxyamino)-2R-isobutyl-3S-thiophenylsulphonyl-methyl-succinyl]-L-phenylalanine-N-methylamide

[4-(N-Hydroxyamino)-2R-isobutyl-3S-phenylsulphonyl-methyl-succinyl]-L-phenylalanine-N-methylamide sodium salt

[4-(N-Hydroxyamino)-2R-isobutyl-3S-(4-(isobutyloxy-carbonylamino)phenyl)thiomethyl-succinyl]-L-phenyl-alanine-N-methylamide

[4-(N-Hydroxyamino)-2R-isobutyl-3S-(4-(N-methyl-N-(tert-butoxycarbonyl)-glycylamino)phenyl)thiomethyl-succinyl]-L-phenylalanine-N-methylamide

or, where appropriate, a salt of such a compound.

10. [4-(N-Hydroxyamino)-2R-isobutyl-3S-(thiophenyl-thiomethyl) succinyl]-L-phenylalanine-N-methylamide, or

[4-(N-Hydroxyamino)-2R-isobutyl-3S-(thiolmethyl) succinyl]-L-phenylalanine-N-methylamide

or a salt thereof.

11. [4-(N-Hydroxyamino)-2R-isobutyl-3S-(thiophenyl-thiomethyl)succinyl]-L-phenylalanine-N-methylamide or a salt thereof.

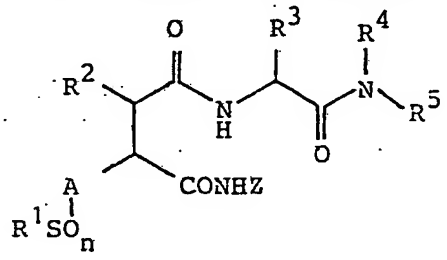
12. A compound as claimed in any one of claims 1 to 11 for use in human or veterinary medicine.

13. The use of a compound as claimed in any one of claims 1 to 11 in the preparation of an agent for use in the management of disease involving tissue degradation and/or in the promotion of wound healing.

14. A pharmaceutical or veterinary formulation comprising a compound as claimed in any one of claims 1 to 11 and a pharmaceutically and/or veterinarily acceptable carrier.

15. A process for preparing a compound of general formula I as defined in claim 1, the process comprising:

(a) deprotecting a compound of general formula II

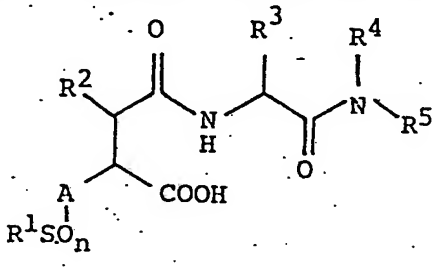


(II)

wherein:

R^1, R^2, R^3, R^4, R^5, A and n are as defined in general formula I and Bn represents a benzyloxycarbonyl group; or

(b) reacting a compound of general formula III



(III)

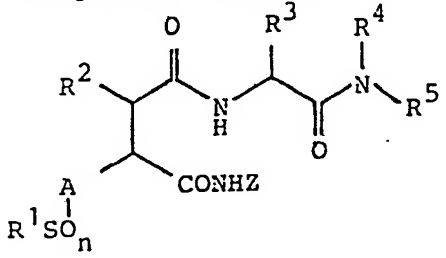
wherein:

R^1, R^2, R^3, R^4, R^5, A and n are as defined in general formula I,

with hydroxylamine or a salt thereof; and

(c) optionally after step (a) or step (b) converting a compound of general formula I into another compound of general formula I.

16. A compound of general formula II

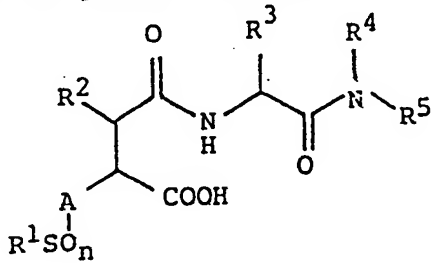


(II)

wherein:

R^1, R^2, R^3, R^4, R^5, A and n are as defined in general formula I and Z represents a protecting group.

17. A compound of general formula III



(III)

wherein:

R^1, R^2, R^3, R^4, R^5, A and n are as defined in general formula I.

INTERNATIONAL SEARCH REPORT

International Application No PCT/GB 89/01399

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all) *

According to International Patent Classification (IPC) or to both National Classification and IPC

IPC[5]: C 07 C 323/62, 323/60, C 07 D 333/34, C 07 C 327/32, 317/50, 313/48, A 61 K 31/13, 31/38

II. FIELDS SEARCHED

Minimum Documentation Searched [7]

Classification System	Classification Symbols
IPC[5]	C 07 C 259/00, 323/00, C 07 D 333/00, C 07 C 327/00, 317/00, 313/00

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched [8]

III. DOCUMENTS CONSIDERED TO BE RELEVANT [9]

Category [10]	Citation of Document, [11] with indication, where appropriate, of the relevant passages [12]	Relevant to Claim No. [13]
A	EP, A, 0236872 (F. HOFFMANN-LA ROCHE) 16 September 1987 see claim 1 cited in the application	1-17
A	EP, A, 0012401 (MERCK & CO. INC.) 25 June 1980 see claim 1 cited in the application	1-17
A	DE, A, 2720996 (E.R. SQUIBB & SONS) 24 November 1977 see claim 1 cited in the application & US, A, 4105789	1-17
A	EP, A, 0274453 (LABORATOIRE ROGER BELLON) 13 July 1988 see claim 1	1-17
A	EP, A, 0214639 (G.D. SEARLE) 18 March 1987 see claim 1	1-17

./.

* Special categories of cited documents: [10]

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report
8th March 1990	17 AVR. 1990
International Searching Authority	**Signature of Authorized Officer**
EUROPEAN PATENT OFFICE	Miss T. TAZELAAR

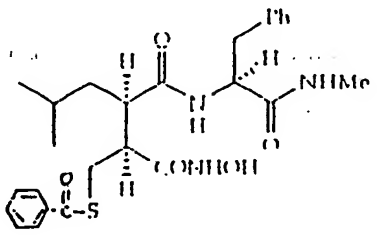
Form PCT/ISA/210 (second sheet) (January 1985)

III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)

Category *	Citation of Document, with indication, where appropriate, of the relevant passages	Relevant to Claim No
	cited in the application & US, A, 4599361 --	
A	Chemical Abstracts, volume 83, no. 7, 18 August 1975, (Columbus, Ohio, US), J.P. Devlin et al.: "Antibiotic actinonin. III. Synthesis of structural analogs of actinonin by the anhydride-imide method", see page 549, abstract 59249e, & J. Chem. Soc., Perkin Trans. I, 1975, (9), 830-41	1-17

Example 6

[4-(N-Hydroxyamino)-2R-isobutyl-3S-(benzoylthiomethyl)-succinyl]-L-phenylalanine-N-methylamide



The title compound was prepared by the method described in Example 1g to give material with the following characteristics

m.p. 227 - 228°

Analysis calculated for $C_{21}H_{31}N_3O_5S$

Requires C 62.50 H 6.66 N 8.41

Found C 62.32 H 6.67 N 8.40

$delta_H$ (250 MHz, $CDCl_3$:D_6DMSO (1:1)) 8.82 (1H, s, NHOH), 8.25 (1H, d, J=8.4Hz, NHOH), 7.87 (2H, dd, J=8.5, 1.1Hz), 7.60 (2H, m, Ar-H and CONH), 7.50 (2H, t, J=8.2Hz), 7.28 (2H, d, J=8.4Hz), 7.16 (2H, t, J=7.2Hz), 7.04 (1H, t, J=8.5Hz), 4.65 (1H, m, $CHCH_2Ph$), 3.06 (1H, dd, J=14.1, 5.0Hz, $CHCH_2Ph$), 2.90 (1H, dd, J=13.9, 10Hz, $CHCH_2Ph$), 2.73 (2H, m SCH_2Ph), 2.65 (3H, d, J=4.7Hz, NHMe), 2.33 (1H, dt, J=11.0, 4.7Hz), 1.51 (1H, t, J=7Hz, CH_2CHMe_2), 1.24 (1H, m, $CHMe_2$), 0.97 (1H, t, J=7Hz, CH_2CHMe_2), 0.84 (3H, d, J=6.5Hz, $CHMe_2$) and 0.79 (3H, d; J=6.5Hz, $CHMe_2$).

ANNEX TO THE INTERNATIONAL SEARCH REPORT
ON INTERNATIONAL PATENT APPLICATION NO. GB 8901399
SA 33118

This annex lists the patent family members relating to the patent documents cited in the above-mentioned international search report.
The members are as contained in the European Patent Office EDP file on 04/04/90
The European Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
EP-A- 0236872	16-09-87	AU-B- 588437	14-09-89
		AU-A- 6990287	17-09-87
		JP-A- 62230757	09-10-87
EP-A- 0012401	25-06-80	AT-T- E6503	15-03-84
		AU-B- 530380	14-07-83
		AU-A- 5346179	19-06-80
		CA-C- 1262684	07-11-89
		JP-A- 55081845	20-06-80
		US-A- 4374829	22-02-83
DE-A- 2720996	24-11-77	US-A- 4105789	08-08-78
		CA-A- 1103259	16-06-81
		FR-A,B 2421874	02-11-79
		GB-A- 1575850	01-10-80
		JP-A- 52136121	14-11-77
		US-A- 4146639	27-03-79
		US-A- 4228184	14-10-80
		US-A- 4153725	08-05-79
		US-A- 4192882	11-03-80
		US-A- 4146641	27-03-79
		US-A- 4207342	10-06-80
		US-A- 4200649	29-04-80
		US-A- 4206232	03-06-80
		US-A- 4192881	11-03-80
		US-A- 4207336	10-06-80
		US-A- 4207337	10-06-80
EP-A- 0274453	13-07-88	FR-A- 2609289	08-07-88
		JP-A- 63258449	25-10-88
EP-A- 0214639	18-03-87	US-A- 4599361	08-07-86
		US-A- 4743587	10-05-88
		AU-B- 588362	14-09-89
		AU-A- 6240886	12-03-87
		JP-A- 62103052	13-05-87

EPO FORM P0479

For more details about this annex : see Official Journal of the European Patent Office, No. 12/82